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NATIONAL INSTITUTES OF HEALTH

ENVIRONMENTAL IMPACT STATEMENT

on

NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

Part One

October 1977

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

Final

ENVIRONMENTAL IMPACT STATEMENT

on

NIH Guidelines for
Research Involving Recombinant DNA Molecules
of June 23, 1976

Prepared by the
Office of the Director
NATIONAL INSTITUTES OF HEALTH
Bethesda, Maryland

October 1977

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Part One

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

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GUIDELINES
FOR
RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

National Institutes of Health
Public Health Service, DHEW
Bethesda, Maryland

( ) Draft (X) Final Environmental Impact Statement

Name of Action

(X) Administrative ( ) Legislative Action

Additional Information

Additional information on the proposed action, including technical documents pertinent to this statement, may be obtained from:

Dr. Donald S. Fredrickson
Director
National Institutes of Health
9000 Rockville Pike
Bethesda, Maryland 20014

Telephone: (301) 496-2433

A copy of the Guidelines for Research Involving Recombinant DNA Molecules is attached. (Appendix D)

SUMMARY SHEET

( ) Draft (x) Final Environmental Impact Statement

Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health

Type of Action: (x) Administrative ( ) Legislative

Brief Description of Action: The action is the release of the NIH Guidelines for Research Involving Recombinant DNA Molecules.

Summary of Environmental Impacts: The primary impact of issuance of the NIH Guidelines is to provide a mechanism for the protection of the laboratory worker, the general public, and the environment from the possible hazards that might result from NIH-supported experiments involving recombinant DNA molecules. These hazards are purely speculative at present. Nevertheless, the Guidelines take cognizance of the possibility of dangers, and call for a number of measures aimed at reducing human and environmental exposure to materials containing recombinant DNA molecules. In the view of the NIH, the level of risk that will result from research in conformance with the Guidelines is acceptably small. With regard to the anticipated benefits of recombinant DNA research, adherence to the Guidelines may postpone their realization. Certain experiments are prohibited; many permissible experiments will be delayed pending availability of suitable containment facilities and certification of appropriate host-vector systems. The text of the EIS discusses the Impact of Issuance of NIH Guidelines, including: (1) Impact on the Safety of Laboratory Personnel and on the Spread of Possibly Hazardous Agents by Infected Laboratory Personnel, (2) Impact on the Environmental Spread of Possibly Hazardous Agents, (3) Cost Impact, and (4) Secondary Impacts. Also discussed is the Impact of Experiments Conducted Under the Guidelines, including: (1) Possible Undesirable Impacts, and (2) Beneficial Impacts of Recombinant DNA Research.

The Following Alternatives Are Analyzed: (A) No Action, (B) NIH Prohibition of Funding of All Experiments with Recombinant DNA, (C) Development of Different Guidelines, (D) No Guidelines but NIH Consideration of Each Proposed Project on an Individual Basis Before Funding, and (E) General Federal Regulation of All Such Research.

Comments Have Been Requested from the Following: A listing of persons and organizations that received the Draft Environmental Impact Statement for comment appears herein as Appendix L. The commentators are listed alphabetically in Appendix K. Chapter VIII contains a detailed response to the comments and explains the transition from Draft EIS to Final EIS.

Date Draft EIS Was Made Available: Notice of availability of the Draft EIS appeared in the Federal Register on September 2, 1976. The entire Draft EIS was published in the Federal Register on September 9, 1976. Although comments were originally requested by October 18, 1976, extensions were given to several commentators at their request. The receipt of formal comments was continued until December 15, 1976.

PREFACE

On June 23, 1976, the Director, National Institutes of Health (NIH), with the concurrence of the Secretary of Health, Education, and Welfare and the Assistant Secretary for Health, issued guidelines that govern the conduct of NIH-supported research involving recombinant DNA molecules. These guidelines appeared in the Federal Register, July 7, 1976 (41 FR 27902 et seq.). The NIH conducted an environmental impact assessment and prepared a Draft Environmental Impact Statement in accordance with the National Environmental Policy Act of 1969. A Draft Environmental Impact Statement on the Guidelines was published in the Federal Register for public comment on September 9, 1976 (41 FR 38425 et seq.).

In recombinant DNA experiments, "genes"--deoxyribonucleic acid (DNA) molecules--from living organisms can be transferred to single cells from completely unrelated organisms. These experiments depend on the ability to join genetic material from different sources and to propagate the resulting elements in single bacterial and animal cells.

The new recombinant DNA technique has resulted in a profound and qualitative change in the field of genetics. Developments in genetic research, particularly in the last 4 years, open avenues to science that were previously inaccessible. Hypotheses and ideas that were not testable can now be rigorously investigated. Understanding of basic biological phenomena has already been enhanced, and the promise of recombinant DNA research for better understanding and improved treatment of human disease is great. Further experimental data will be required to delimit the benefits that may be derived through this technique.

There is also a possible risk that microorganisms with foreign genes could cause disease or adversely affect the environment if they should escape from the laboratory and infect human beings, animals, or plants. Many recombinant DNA experiments have been conducted throughout the world during the past 5 years and are continuing. To date, no known hazardous organism has been produced in this work. Thus, the risk of converting harmless organisms to harmful ones by recombinant DNA experiments remains hypothetical. Further work will eventually determine the limits of these hypothetical risks.

A Federal Interagency Committee on Recombinant DNA Research recommended in March 1977 that legislation be passed to extend the standards of the NIH Guidelines to all recombinant DNA activities in the public and private sectors. On the basis of these recommendations, Joseph A. Califano, Jr., Secretary of Health, Education, and Welfare,

had legislation developed, and an Administration bill was introduced in Congress. Congressional hearings were held and separate bills are now being considered by the House of Representatives and the Senate to convert the Guidelines to standards for regulatory purposes. It should be noted that certain types of experiment covered in the Guidelines would no longer be subject to regulation in the bills.

The NIH Guidelines set strict conditions for the conduct of NIH-supported research in this area. They prohibit certain types of experiments and require special safety conditions for other types. The provisions are designed to afford protection with a wide margin of safety to workers, the public, and the environment.

The text of this Final Environmental Impact Statement discusses the Impact of Issuance of NIH Guidelines, under the following sections: (1) Impact on the Safety of Laboratory Personnel and on the Spread of Possibly Hazardous Agents by Infected Laboratory Personnel, (2) Impact on the Environmental Spread of Possibly Hazardous Agents, (3) Cost Impact, and (4) Secondary Impacts. Also discussed is the Impact of Experiments Conducted Under the Guidelines, including: (1) Possible Undesirable Impacts and (2) Beneficial Impacts of Recombinant DNA Research.

The Final EIS has been substantially modified in both text and appendices to take into account the comments received on the Draft Environmental Impact Statement. In addition to the modifications of the EIS in response to comments, modifications have also been based on new knowledge and developments since the Draft EIS was prepared in the summer of 1976.

Many of the issues raised by the commentators on the Draft EIS were previously considered in developing the NIH Guidelines for Research Involving Recombinant DNA Molecules. In drafting an impact statement, however, the NIH has given further consideration to these issues and has provided an opportunity for the public to comment from an environmental perspective. This perspective has been very helpful in NIH's review of policies and procedures on recombinant DNA research.

TABLE OF CONTENTS

Page

PART ONE: 'FINAL ENVIRONMENTAL IMPACT STATEMENT

I. Foreword 1

II. Authority 7

III. Objective of the NIH Action 8

IV. Background 9

A. Description of the Recombinant DNA Experimental Process 9

B. Events Leading to the Development of Guidelines 15

C. Description of Issues Raised by Recombinant DNA Research 23

1. Possible Hazardous Situations 23
2. Expected Benefits of Recombinant DNA Research. . 31
3. Long-Range Implications. 36
4. Possible Deliberate Misuse 38

V. Description of the Action 44

A. General Application of the Guidelines 44

B. Physical Containment Requirements. 47

C. Biological Containment Requirements 53

D. Combination of Physical and Biological Containment in Practice 55

E. Compliance with the Guidelines 62

VI. Description of Alternatives 65

A. No Action 65

B. NIH Prohibition of Funding of All Experiments with Recombinant DNA 66

C. Development of Different Guidelines. 67

Page

1. Levels of Physical Containment 67

2. The Recommendation that All Permissible Recombinant DNA Experiments Be Conducted in National Facilities. 69

3. Experiments Prohibited at this Time 70

D. No Guidelines but NIH Consideration of Each Proposed Project on an Individual Basis Before Funding 83

E. General Federal Regulation of All Such Research. . . . 83

VII. Environmental Impact of the Guidelines 89

A. Impact of Issuance of NIH Guidelines 89

1. Impact on the Safety of Laboratory Personnel and on the Spread of Possibly Hazardous Agents by Infected Laboratory Personnel 90

2. Impact on the Environmental Spread of Possibly Hazardous Agents 96

3. Cost Impact. 97

4. Secondary Impacts 98

B. Impact of Experiments Conducted Under the Guidelines 99

1. Possible Undesirable Impacts 99

2. Beneficial Impacts of Recombinant DNA Research 109

VIII. Responses to Comments on the Draft Environmental Impact Statement 113

PART TWO: APPENDICES

BACKGROUND

Appendix		Page
A.	Glossary	A--1
B.	Suggested References for Additional Reading	B--1
C.	Documents Describing the Implementation of the Guidelines	C--1
D.	"NIH Guidelines for Research Involving Recombinant DNA Molecules," including "Decision of the Director, National Institutes of Health, To Release Guidelines for Research on Recombinant DNA Molecules"	D--1
E.	"A Summary of the National Institutes of Health (USA) Guidelines for Recombinant DNA Research" by Maxine F. Singer	E--1
F.	Institutions with Biohazards Committees (list)	F--1
G.	Ongoing NIH-Supported Projects Involving Recombinant DNA	G--1
H.	Certification of EK2 Host-Vector Systems	H--1
	I. Report to the Recombinant Advisory Committee from the Working Group on Safer Hosts and Vectors: Lambda Phage Systems	H--1
	II. Instructions to Investigators Concerning Data To Be Submitted on Host-Plasmid Systems Proposed for EK2 Certification	H--6
	III. Certified EK2 Host-Vector Systems	H--10
I.	Interagency Committee on Recombinant DNA Research: Membership	I--1
J.	Interim Report of Interagency Committee	J--1

Appendix | Page

COMMENTS

K. Public Comments on Draft EIS of September 9, 1976 . . . . . K--1

L. Partial List of Persons and Organizations to Whom the Draft EIS Was Sent for Comment L--1

RECENT DEVELOPMENTS

M. Letter from S.L. Gorbach to D.S. Fredrickson, reporting on a Workshop on Studies for Assessment of Potential Risks Associated with Recombinant DNA Experimentation (Falmouth, Mass., June 20-21, 1977) M--1

N. "A Bill To regulate activities involving recombinant deoxyribonucleic acid" (S.1217) N--1

O. Excerpts from an article (in press) by R. Freter, Real and Imagined Dangers of Recombinant DNA Technology: The Need for Expert Evaluation in a monograph The Recombinant DNA Debate. O--1

P. Excerpts from an article by R. Holliday (1977): Should Genetic Engineers Be Contained? P--1

Q. Article by Carl Cohen (1977): When May Research Be Stopped? Q--1

I.
FOREWORD

Recent developments in molecular genetics, particularly in the last 4 years, open avenues to science that were previously inaccessible. In the "recombinant DNA" experiments considered here, genes--deoxyribonucleic acid (DNA) molecules--from virtually any living organism can be transferred to single cells from certain completely unrelated organisms. These experiments depend on the ability to join together genetic material from two different sources and then to propagate the resulting hybrid elements in single bacterial and animal cells.

For example, genes have been transferred from bacteria of one species to bacteria of another. And genes from amphibians or insects have been introduced into the bacterium Escherichia coli. If the recipient cell is then allowed to multiply in the laboratory, it will propagate these newly acquired genes as part of its own genetic complement.

It should be understood that genetic recombination itself (other than what is referred to as "recombinant DNA") is a well-known phenomenon and has been occurring in nature for millions of years. The recombining of genetic material (DNA) is an integral part of sexual reproduction.

The new recombinant DNA technique has resulted in a profound and qualitative change in the field of genetics. Hypotheses and ideas that were not previously testable can now be rigorously investigated. Understanding of basic biological phenomena has already been enhanced and will certainly be enlarged as experiments proceed. The promise of recombinant DNA research for better understanding and improved treatment of human disease is also great. Further experimental data will be required to delimit the benefits that may be derived through these techniques.

There is also a possible risk that the properties of a microorganism with foreign genes might be altered so that it could cause disease or adversely affect the environment if it should escape from the laboratory and infect human beings, animals, or plants. Throughout the world many recombinant DNA experiments have been conducted during the past 5 years and are continuing today. To date, no known hazardous organism has been produced in this work. Thus, the risk of converting harmless organisms to harmful ones by recombinant DNA experiments remains hypothetical, but cannot be ignored. Further work will eventually determine the limits of these hypothetical risks. Meanwhile, it is assumed that hazards of varying degrees may exist and that some precautions are justified to minimize the probability of their occurrence.

On June 23, 1976, the Director of the National Institutes of Health, Donald S. Fredrickson, M.D., released Guidelines governing the conduct of NIH-supported research on recombinant DNA molecules (see Appendix D). Promulgation of these Guidelines followed 2 years of intensive discussion and debate within the scientific community and NIH itself, with public participation, concerning the hazards of such research and the best means for dealing with known hazards and averting the hypothetical risks. The Guidelines prohibit certain kinds of recombinant DNA experiments, which include virtually all the known hazards--for example, those involving known infectious agents. For those experiments that are permitted, the Guidelines specify safety precautions and conditions designed to protect the health of laboratory workers, the general public, and the environment against hypothetical hazards.

The issuance of Guidelines establishing conditions and precautions with respect to recombinant DNA experiments is viewed by NIH as within the category of a Federal action that may significantly affect the quality of the human environment. Hence, the NIH Director ordered the publication of this statement--first in draft (Federal Register, September 9, 1976) and now in final--pursuant to the National Environmental Policy Act (NEPA).

Although NEPA assumes that such Federal actions will not be taken until the NEPA procedures are completed, the Director of NIH concluded that the public interest required immediate issuance of the Guidelines, rather than deferral for the months that would be required for completion of the NEPA process. This was because experiments utilizing recombinant DNA technology were proceeding in various laboratories throughout the country with only general and purely voluntary restrictions. Therefore, the escape of potentially hazardous organisms was more likely in the absence of NIH action. Further, prompt issuance of the Guidelines was believed necessary in order to promote their acceptance by scientists in the United States and abroad who do not come under the purview of NIH.

Thus the issuance of the Guidelines is an action designed to protect the environment from possible untoward effects of recombinant DNA experiments. Undeniably, the conduct of recombinant DNA experiments in conformance with the Guidelines results in less risk to the environment than the conduct of such experiments without guidelines. Evaluation of the protection afforded by the Guidelines depends on the assessment of risks related to the experiments, necessitating consideration of the experiments as well as the Guidelines.

Issuance of and compliance with the Guidelines decreases the chance that NIH-assisted recombinant DNA research will have a negative effect on the environment. To the extent that the Guidelines are used as a model of safe research practice for recombinant DNA research that is not NIH-assisted, the risk of negative environmental effect will be further reduced. Confidence in the Guidelines is derived mainly from a consensus of the judgments of knowledgeable scientists who have been consulted. There is also an experimential base that is relevant. This is the fact that laboratory scientists have worked for about 100 years with various agents identified as capable of causing disease without any known adverse impact on the environment. The confidence of the scientists who have prepared and critically reviewed these guidelines is based on two premises. First, it is believed that the containment measures specified in the Guidelines make the escape of potentially harmful recombinant organisms into the environment highly improbable. Second, it is believed that even if an experiment performed in accordance with the Guidelines does result in accidental release of recombinant organisms, adverse effects will either not occur or not be serious.

In the absence of an adequate base of data derived from either experiments or experience, it must be recognized that future events may not conform to these judgments. There is some statistical probability that recombinant organisms will find their way into the environment either from experiments under NIH auspices or from the activities of others. It is not difficult to construct hypothetical scenarios in which injury could result. Although the possibility of significant environmental consequences is speculative, the chance of an event that could cause severe injury, however low the probability, must be treated as a possible adverse impact on the environment.

The NIH Guidelines, while designed to ensure the safety of NIH-supported researchers, the general public, and the environment, also serve as a model for other laboratories throughout the world, thereby promoting environmental protection not otherwise achievable through the Federal Government. And it should be emphasized that the experiments which are the subject of the Guidelines are expected to lead ultimately to an increase of knowledge and the advancement of medicine and other sciences in ways that should profoundly benefit mankind.

The Director of NIH believes that the NEPA review will focus attention on the important issues involved, in the interest of gaining the understanding and views of the broadest possible segment of the American people. In issuing the Guidelines, the NIH Director pointed out that they will be subject to continuous review and modification in the light of changing circumstances. Constructive modification could result from information received during the NEPA process.

Notice of availability of the Draft Environmental Impact Statement appeared in the Federal Register of September 2, 1976, and was announced in a press release and in the NIH Guide to Grants and Contracts. NIH sent out over 2,000 copies of the full Draft for review following its publication on September 9. A partial list of addressees is given in Appendix L. All 38 comments received are reproduced in their entirety in Appendix K, and our analytic response to the comments appears as Chapter VIII. (The organization of the Draft EIS and the Final EIS are identical, except for the addition of Chapter VIII and Appendices E thru Q in the latter document.)

An index at the beginning of Appendix K lists the 38 commentators alphabetically, identifies them, and cites the sections in Chapter VIII where our responses to the commentators are given. Similarly, Chapter VIII cites (in brackets) documents of commentators addressing the point in question. Thus the index to Appendix K (page K--2) serves as a bridge between Appendix K and Chapter VIII and between these and the body of the EIS (Chapters I thru VII).

Based on the comments and our response, the text of the EIS has been modified in numerous places. If no modification was called for in response to a certain comment, this is explained in Chapter VIII. If we concluded that the EIS should be modified, this is discussed in Chapter VIII and the appropriate modifications have been made in the body of the EIS.

In addition to modifications of the EIS in response to comments received, we have also made modifications based on new knowledge and developments. The Draft EIS was prepared in the summer of 1976. That statement is now being completed in the fall of 1977. During this year, many recombinant DNA experiments have been performed and much discussion and debate concerning recombinant DNA has taken place both in the scientific community and among the public. Some of the benefits of the research predicted a year ago--namely, important advances in knowledge of basic genetics--have now been realized. On the other hand, none of the hypothetical risks has been demonstrated.

Improved systems for biological containment of recombinant DNA have been developed, and extensive data on the properties of these systems have been accumulated. The data have been carefully scrutinized by the Recombinant Advisory Committee, which has found the systems to be extremely safe. They have been so certified by NIH. Medical microbiologists have looked closely at the question whether E. coli K-12 (the bacterium used in most recombinant DNA experiments) can be changed into a pathogen by the insertion of recombinant DNA, and have concluded that it is virtually impossible. Among the community of experts in this field, presented with these new data, an earlier consensus that the NIH Guidelines were very conservative has tended to shift to the view that they are now excessively so and therefore unnecessarily restrictive (1-7).

A noteworthy feature of recombinant DNA research is that few if any of the NIH grants or contracts involving its use support such research per se. The new technology has evolved in the search for better methods to study control of gene function, a fundamental key to numerous biological and medical problems. Recombinant techniques are but a few of many techniques employed in such research. They are thus analogous to the use of radioisotopes. And like radioisotopes, they are used world-wide and are not confined to NIH-supported research in this country or abroad.

In light of the uncertainties surrounding the benefits and hypothetical risks of the use of recombinant DNA technologies, NIH believes it important to have a recombinant DNA "program" in the interest of providing and implementing safety guidelines and of assessing the potential of the research for good and harm.

References

(1) Watson, J.D. (1977). In Defense of DNA. The New Republic:11, June 25, 1977.

(2) Curtiss III, Roy (1977). Letter to Donald S. Fredrickson, April 12, 1977.

(3) Hogness, David (1977). Letter to DeWitt Stetten, January 26, 1977.

(4) Falkow, Stanley (1977). Letter to DeWitt Stetten, June 15, 1977.

(5) Norman, Colin (1977). Genetics, Full Circle. Nature 267:748, June 30, 1977.

(6) Gorbach, Sherwood L. (1977). Letter to Donald S. Fredrickson, July 14, 1977. (See Appendix M.)

(7) Philipson, L., and P. Tiollais (1977). Rational Containment on Recombinant DNA. Nature 268:90-91, July 14, 1977.

II.
AUTHORITY

The Federal action discussed in this document is taken under the authority of Title III of the Public Health Service Act: General Powers and Duties of Public Health Service; Part A--Research and Investigation; Sections 301 and 307 [42 U.S.C. 241 and 242(1)].

III.
OBJECTIVE OF THE NIH ACTION

The action--release of the NIH Guidelines--is intended to allow the benefits of NIH-sponsored recombinant DNA research to accrue while protecting laboratory workers, the general public, and the environment from infection by possibly hazardous agents that may result from the research. The Guidelines are meant to ensure that NIH-supported experiments involving recombinant DNA molecules are carried out under conditions and safeguards that minimize the possibility of the harmful exposure of any human being or other component of the environment to any possibly hazardous product of such experiments.

It is NIH policy that all work supported by NIH, either in its own laboratories or through grants or contracts to various organizations, must be carried out according to the Guidelines. As part of this objective, the Guidelines describe procedures that will be used to ensure implementation. A further objective of establishing the Guidelines is to influence, to the extent possible, other Federal, non-Federal, and foreign organizations in their efforts to ensure that recombinant DNA experiments will be carried out with minimal risk.

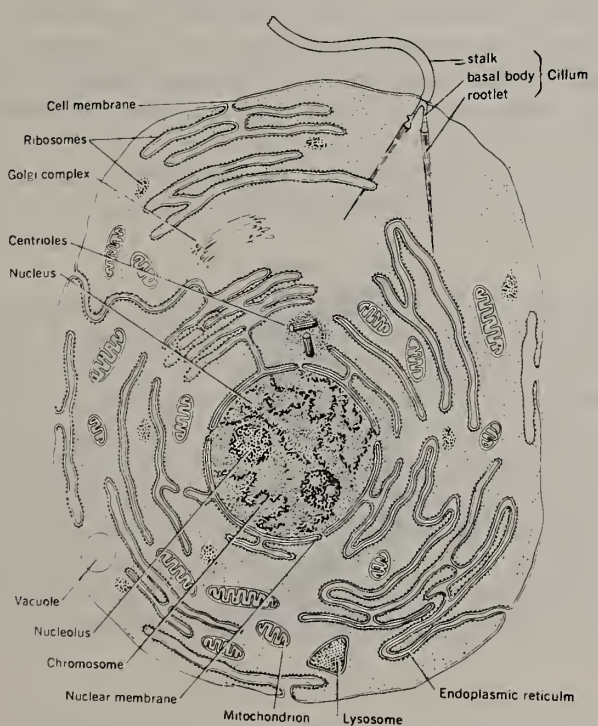
IV.
BACKGROUND

A. Description of the Recombinant DNA Experimental Process*

All living things, from subcellular particles to higher organisms, contain the specific information needed for their reproduction and functions. The basic source of this information is deoxyribonucleic acid (DNA), which is the principal substance of the genes, the units of heredity. Each gene directs the synthesis of a different protein, and the diversity in structure and function of proteins accounts for the differences between all living things.

Each cell of an organism is composed of various organized structures, several of which contain DNA. Figure IV-1 illustrates a typical eukaryotic (i.e., nucleated) cell. Bacterial cells are much less complex, showing fewer organelles and no organized nucleus.

Figure IV-1



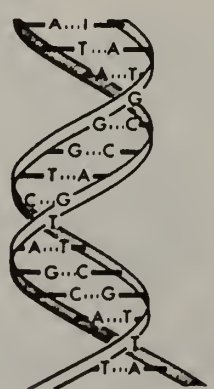
*The reader is referred to references 1, 2, 3, 4, and 5 at the end of Section IV for additional information on the scientific concepts introduced in this section. References 3 and 5 should be especially informative to nonscientists.

DNA plays two roles: (1) provides information for the reproduction, growth, and functions of the cell, and (2) preserves and directs replication of this information and transfers it to the offspring. These two roles of DNA are common to animals, plants, single-cell organisms, and many viruses. The DNA of cells is mainly found in organized structures called chromosomes.

Intracellular DNA also occurs outside of the chromosomes as separately replicating molecules. Such DNA molecules include the plasmids, found in bacteria; the DNA of chloroplasts, common to green plants; and the DNA of mitochondria, the energy-producing units of the cells of complex organisms. These DNAs, while not strictly part of the inherent genetic make-up of a cell, help define the cell's functional capability. Another type of DNA commonly found in cells is the DNA of infecting viruses.

During the past 30 years the structure of DNA molecules has been studied intensively, and it can now be described in much detail. The molecule may be compared to a long but twisted stepladder with thousands to millions of rungs (a short piece of DNA is shown in Figure IV-2). The sides of the ladder are formed of sugar molecules (deoxyribose) attached end to end through phosphate groups. At right angles to each sugar molecule is one of four possible bases--adenine, guanine, thymine, and cytosine. The precise sequence of these bases, the rungs of the ladder, codes the information content. The "reading" of the code contained in the sequence of bases results in the formation of proteins, which in turn carry out most of the essential functions of the cell.

Figure IV-2



A gene is a portion of the DNA molecule which codes for the manufacture of a single protein. In higher organisms, much of the DNA may not serve as genes in this sense, but may regulate the activity of nearby genes. It is possible to break open cells and isolate DNA, free of other cellular constituents.

The genetic make-up of cells and organisms is basically stable, thus ensuring the proper functioning of the organism and its offspring. This stability is not absolute, however, and while changes are relatively rare, they occur frequently enough to have profound effects on the nature of living things. Such changes, called "mutations," are basic to genetics and essential to evolution. From a chemical point of view, all mutations can be described as changes in the structure of DNA.

Changes of several different types are known to occur. If one of the bases in the portion of the DNA molecule coding for a given gene is changed to another base, that gene is thereby a mutant gene; and the protein manufactured by it will usually differ from the protein manufactured by the original gene. Similarly, the protein will be different (or perhaps will not be made at all) if a series of bases in the original gene are simply deleted, or if a new series of bases is inserted within the original sequence of the gene. The latter two types of mutation--deletions and insertions--occur in connection with the phenomenon known as crossing-over, or recombination.

Recombination in Nature. This phenomenon has long been known to occur within cells of a given species of both simple, single-celled organisms and more complex animals and plants. Genetic information, or rather segments of DNA molecules bearing information, can be exchanged between chromosomes and between different regions of a given chromosome. Such genetic recombination contributes importantly to biological diversity.

Recombination results from breaking and then joining of two separate DNA molecules. The two molecules involved may be from the chromosomes of the organism itself, or one or both of them may be the DNA of a plasmid or virus. Generally, these events have been observed only within a given species: they may involve the DNA of the single organism and that of plasmids or viruses that infect only that species. For example, genetically determined resistance to antibiotics is transferred readily between free-living microorganisms. Recombination between different species of single-celled microorganisms has also been described in a limited number of cases. It is not known how widespread such interspecies recombination is in nature.

As far as is known, in order for recombination to occur naturally, the two DNA molecules must be within the same cell. Plasmids and viruses can be transferred from cell to cell by natural mechanisms that have been studied in some detail. Transfer of plasmids from one cell to another requires the physical contact of the two cells. Viruses, on the other hand, may be transferred without cell contact; their genetic material is protected from the effects of the environment by a coat of proteins. In addition, it is possible under some circumstances for DNA segments, free in a solution, to enter a cell and undergo recombination therein. This process has been called transformation (6). Thus far, transformation of this type has only been detected in a small number of species of bacteria; it has not been observed with more complex organisms.

In complex organisms that reproduce sexually, each offspring receives DNA from its parents. For example, every human being receives half of his complement of DNA from a sperm cell and half from an egg cell. In each generation the human genetic material is recombined.

Recombination in the laboratory. In the recombinant DNA experiments that are the subject of this Statement and the accompanying Guidelines, DNA is first isolated from two different cell types. Each DNA is then broken into segments. Each segment may contain one or more genes, or it may contain a portion of the DNA that lacks functional genes. The breaking can be accomplished by several means, including bacterial enzymes (e.g., restriction endonucleases), which cut the DNA in such a way that the chemical structure at the ends of the segments permits interchangeable rejoining when the two different DNAs are mixed. Alternatively, the necessary structures at the ends of the DNA segments may be introduced after other types of breakage. In this way single DNA molecules containing portions of the two different DNAs are constructed.

The DNA recombined in these experiments can be derived from widely divergent sources. DNA from one of the sources may serve as a carrier, or vector, for the insertion of the recombined DNA into a cell, or host. The vector may be a plasmid, usually derived from the same species as will serve as the host, or a virus. The DNA to be inserted is called the "foreign" DNA. When a large mixture of DNA fragments from the foreign source is used in the joining, the experiment is referred to as a "shotgun" experiment. In other instances, a particular DNA fragment of interest will be purified and then joined to the vector.

From a growth culture of the host cells, those containing the DNA fragment of particular interest are selected and allowed to multiply. The resulting population of identical cells is called a "clone."* In some experiments the DNA will be extracted from the cells for study; in others, the properties of the cells themselves will be investigated.

In the experiments discussed in the Guidelines, the host cells are generally single-cell microorganisms such as bacteria, or animal or plant cells that were originally obtained from living tissue but are grown as single cells under special laboratory conditions.

The process of producing recombinant DNA molecules and introducing them into cells is illustrated in Figure IV-3.

Recombinant DNA technology represents a method that is applicable to many areas of biological research. Essentially, it represents a new tool.

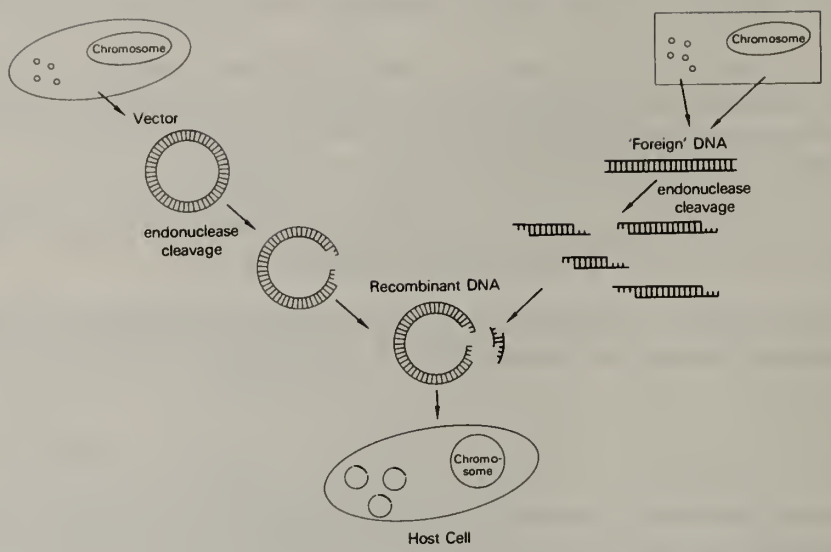
Indeed, investigations carried out under a variety of NIH Institutes and programs, and many non-NIH-supported studies in the United States and throughout the world as well, are utilizing this technique, much as a new instrument may be applied to studying many different things. Diverse areas of biological research to which recombinant DNA experiments are already being applied include studies of--

- bacterial enzymes and metabolism,
- the synthesis of hormones (e.g., insulin),
- the reproduction of viruses,
- the organization of chromosomes, and
- the structure and regulation of genes coding for the synthesis of globin (hemoglobin), immunoglobulins, histones, and ovalbumin (egg protein).

Thus, except with the aim of improving the technology, investigations of "recombinant DNA" per se will not occur. Rather, recombinant DNA technology will be used, where applicable, as an additional tool for increasing understanding of normal and abnormal biological processes.

*The word "clone" means a population of cells derived from a single cell. In recombinant DNA experiments, it refers to a population of single cells with identical DNA content--thus the term "molecular cloning." Molecular cloning is therefore different from the growth of whole organisms (e.g., frogs) from single cells, a process which is also called "cloning."

Figure IV-3



The cell represented at the upper left contains chromosomal DNA and several separately replicating DNA molecules. The nonchromosomal DNA molecules can be isolated from the cell and manipulated to serve as vectors (carriers) for DNA from a foreign cell. Most DNA molecules used as vectors are circular. They can be cleaved, as shown, by enzymes (restriction endonucleases) to yield linear molecules with rejoinable ends.

At the upper right is another cell, represented here as a rectangle. It serves as the source of the foreign DNA to be inserted into the vector. This DNA can also be cleaved by enzymes. The rectangular cell could be derived from any living species, and the foreign DNA might contain chromosomal or nonchromosomal DNA, or both.

In the next steps, the foreign DNA fragment is mixed and combined with the vector DNA, and the recombinant DNA is reinserted into a host cell. In most experiments this host cell will be of the same species as the source of the vector. The recipient cells are then placed under conditions where they grow and multiply by division. Each new cell will contain recombinant DNA.

B. Events Leading to Development of Guidelines

On June 23, 1976, the Director, NIH, released "National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules" (see Appendix D). This action was approved by the Secretary of Health, Education, and Welfare and the Assistant Secretary for Health. The Guidelines establish carefully controlled conditions for the conduct of experiments involving the insertion of recombinant genes into organisms, such as bacteria.

For about 3 years prior to the time the Guidelines were issued, recombinant DNA experimentation was carried out in the United States and abroad. Indeed, from the time scientists first expressed concern about the work, it has proceeded subject to restrictions adopted along the way, and much has been learned through use of the new technology. At no time has this scientific tool been banned completely. The heart of the controversy surrounding recombinant DNA is to define appropriate limits for application of the technology, which is new and might involve unknown risks. The chronology of events leading to the present Guidelines and the decision to release them is as follows.

It was some of the scientists engaged in recombinant DNA research who called for a voluntary deferral of certain kinds of experiments, in order to provide time to assess the risks and devise appropriate guidelines. This limited voluntary deferral came to be called a "moratorium." The capability to perform DNA recombinations, and the possibility that some such experiments might be hazardous, had become apparent at the Gordon Research Conference on Nucleic Acids in July 1973. Those in attendance voted to send an open letter to Dr. Philip Handler, President of the National Academy of Sciences, and to Dr. John R. Hogness, President of the Institute of Medicine, NAS. The letter, appearing in Science (7), suggested that the Academy "establish a study committee to consider this problem and to recommend specific actions or guidelines, should that seem appropriate."

In response, NAS formed a committee, and its members published another letter in Science in July of 1974 (8). Under the title "Potential Biohazards of Recombinant DNA Molecules," the letter proposed:

> First, and most important, that until the potential hazards of such recombinant DNA molecules have been better evaluated or until adequate methods are developed for preventing their spread, scientists throughout the world join with the members of this committee in voluntarily deferring the following types of experiments.

> Type 1: Construction of new, autonomously replicating bacterial plasmids that might result in the introduction of genetic determinants for antibiotic resistance or bacterial toxin formation into bacterial strains that do not at present carry such determinants; or construction of new bacterial plasmids containing combinations of resistance to clinically useful antibiotics unless plasmids containing such combinations of antibiotic resistance determinants already exist in nature.
>
> Type 2: Linkage of all or segments of the DNA's from oncogenic or other animal viruses to autonomously replicating DNA elements such as bacterial plasmids or other viral DNA's. Such recombinant DNA molecules might be more easily disseminated to bacterial populations in humans and other species, and thus possibly increase the incidence of cancer or other diseases.

Second, plans to link fragments of animal DNAs to bacterial plasmid DNA or bacteriophage DNA should be carefully weighed. . . .

Third, the Director of the National Institutes of Health is requested to give immediate consideration to establishing an advisory committee charged with (i) overseeing an experimental program to evaluate the potential biological and ecological hazards of the above types of recombinant DNA molecules; (ii) developing procedures which will minimize the spread of such molecules within human and other populations; and (iii) devising guidelines to be followed by investigators working with potentially hazardous recombinant DNA molecules.

Fourth, an international meeting of involved scientists from all over the world should be convened early in the coming year to review scientific progress in this area and to further discuss appropriate ways to deal with the potential biohazards of recombinant DNA molecules.

Compliance with the voluntary deferral of the experiments listed as Type 1 and Type 2 in the 1974 letters appears to have been essentially universal within the international scientific community. The evidence for this conclusion comes both from the informal communications network among scientists in relevant fields and from inspection of publications appearing in scientific journals during and subsequent to the period in which the voluntary deferral was in force.

On October 7, 1974, the NIH Recombinant DNA Molecule Program Advisory Committee (hereafter "Recombinant Advisory Committee") was established to advise the Secretary of HEW, the Assistant Secretary for Health, and the Director of NIH "concerning a program for developing procedures which will minimize the spread of such molecules within human and other populations, and for devising guidelines to be followed by investigators working with potentially hazardous recombinants."*

Asilomar. The international meeting proposed in the Science article (8) was held in February 1975 at the Asilomar Conference Center, Pacific Grove, California. It was sponsored by the National Academy of Sciences and supported by the National Institutes of Health and the National Science Foundation. One hundred and fifty people attended, including 52 foreign scientists from 15 countries, 16 representatives of the press, and 4 attorneys.

*The use of the term "program" in the name of this Committee and in its charter has been confusing. It refers to a program for developing procedures that will minimize the spread of recombinant DNA molecules--that is, the safety aspects of recombinant DNA research--and not to a program of recombinant DNA research itself. Recombinant DNA technology is essentially a tool used in many programs, funded from many sources. In this sense, it is analogous to a new instrument or radioisotopes. For example, there are NIH-funded recombinant DNA projects as part of the genetics program of the National Institute of General Medical Sciences, as part of the microbiology and infectious diseases program of the National Institute of Allergy and Infectious Diseases, as part of the biological research program of the National Cancer Institute, and as part of the intramural research program of the National Institute of Child Health and Human Development.

The conference reviewed progress in research on recombinant DNA molecules and discussed ways to deal with the potential biohazards of the work. Participants felt that most experiments on construction of recombinant DNA molecules should proceed, but that appropriate containment should be utilized until hypothetical hazards could be better assessed. The conference made recommendations for matching levels of containment with levels of presumed potential hazard for various types of experiment. It also recommended against the performance of certain experiments at that time. Some of these involved the intrinsic hazard of using organisms known to cause disease. Others involved hypothetical risks that seemed greater than reasonably necessary to permit knowledge about the new techniques to be obtained cautiously and prudently. The proscribed experiments included the cloning of recombinant DNAs derived from highly pathogenic organisms, of DNA containing toxin genes, and large-scale experiments (more than 10 liters of culture) if recombinant DNAs are able to make products potentially harmful to man, animals, or plants.

A report on the conference was submitted to the Assembly of Life Sciences, National Research Council, NAS, and approved by its Executive Committee on May 20, 1975. A summary statement of the report (9) was published in Science, Nature, and the Proceedings of the National Academy of Sciences. The report noted that "in many countries steps are already being taken by national bodies to formulate codes of practice for the conduct of experiments with known or potential biohazard. Until these are established, we urge individual scientists to use the proposals in this document as a guide."

Again, while many recombinant DNA experiments not subject to the moratorium continued after Asilomar, there is substantial evidence that the Asilomar recommendations were voluntarily observed throughout the world. In this regard it is noteworthy that the experiments recommended for deferral in the Asilomar report (9) differ from those mentioned in the 1975 report of the NAS committee (see above and reference 8). Insofar as they relate to experiments with antibiotics, the experiments described as Type 1 by the NAS committee were not among those recommended for deferral by the Asilomar report. The Asilomar report stated: "Experiments extending the range of resistance of established human pathogens to therapeutically useful antibiotics or disinfectants should be undertaken only under moderate or high risk containment, depending upon the virulence of the organisms involved."

Similarly, while the experiments described as Type 2 by the NAS committee report included linkage of total DNAs or segments of the DNAs from any animal virus, oncogenic (cancer-causing) or not, the experiments to be deferred under the Asilomar recommendations included only those in which the viral DNA was derived from highly pathogenic viruses as listed by the Center for Disease Control (classes 3, 4, and 5). For those viruses that the Asilomar report considered permissible for use in recombinant DNA experiments, the report recommended use of bacterial vector-host systems with demonstrably restricted growth capabilities outside the laboratory, together with moderate-risk physical containment facilities. With rigorously purified segments of DNA known not to contain genes for oncogenic transformation, existing host-vector systems--that is, not specially designed systems--were deemed permissible.

From May 1975, when the Asilomar report was published, until the promulgation of the NIH Guidelines in June 1976, the recommendations of the Asilomar Conference served as guidance for NIH-supported investigators. In general, this was on a voluntary basis. In the winter of 1975-76, the various Institutes of the NIH wrote to grantees involved in recombinant DNA and related research calling attention to the potential hazards of certain experiments, and the need for caution, and indicating that certain experiments were not to be carried out at all (see representative letter in Appendix D to Appendix C). These prohibitions included those in the Asilomar recommendations, but also included experiments designated as Type 1 by the 1975 NAS Committee letter. Within the restrictions of the Asilomar guidelines, an increasing number of NIH grantees, contractors, and intramural scientists were utilizing the new procedures. Methods were improved. In addition, serious work on the development of safer hosts and vectors was initiated in several laboratories.

Recombinant Advisory Committee. The NIH Recombinant Advisory Committee held its first meeting in San Francisco immediately after the Asilomar conference in February 1975. It proposed that NIH use the recommendations of the Asilomar conference as guidelines for research until the committee had an opportunity to elaborate more specific guidelines, and that NIH establish a newsletter for informal distribution of information. NIH accepted these recommendations.

At the second meeting, held on May 12-13, 1975, in Bethesda, Maryland, the committee received a report on biohazard-containment facilities in the United States and reviewed a proposed NIH contract program for the construction and testing of microorganisms that would have very limited ability to survive in natural environments and would thereby limit any possible hazards. A subcommittee chaired by Dr. David Hogness was appointed to draft guidelines for research involving recombinant DNA molecules, to be discussed at the next meeting.

The NIH committee, beginning with the draft guidelines prepared by the Hogness subcommittee, prepared proposed guidelines for research with recombinant DNA molecules at its third meeting, held on July 18-19, 1975, in Woods Hole, Massachusetts.

Following this meeting, many letters were received which were critical of the guidelines. The majority of critics felt that they were too lax, others that they were too strict. The committee reviewed all letters, and a new subcommittee, chaired by Dr. Elizabeth Kutter, was appointed to revise the guidelines.

A fourth committee meeting was held on December 4-5, 1975, in La Jolla, California. For this meeting a "variorum edition" had been prepared, comparing line-for-line the Hogness, Woods Hole, and Kutter guidelines. The committee reviewed these, voting item-by-item for their preference among the three variations and, in many cases, adding new material. The result was the "Proposed Guidelines for Research Involving Recombinant DNA Molecules," which were referred to the Director, NIH, for a final decision in December 1975.

Public Meeting of the NIH Director's Advisory Committee. The Director of the National Institutes of Health called a special meeting of the Advisory Committee to the Director to review these proposed guidelines. The meeting was held at NIH, Bethesda, on February 9-10, 1976. The Advisory Committee is charged to advise the Director, NIH, on matters relating to the broad setting--scientific, technological, and socioeconomic--in which the continuing development of the biomedical sciences, education for the health professions, and biomedical communications must take place, and to advise on their implications for NIH policy, program development, resource allocation, and administration. The members of the committee are knowledgeable in the fields of basic and clinical biomedical sciences, the social sciences, physical sciences, research, education, and communications. In addition to current members of the committee, the Director, NIH, invited a number of former committee members as well as other scientific and public representatives to participate in the special February session.

The purpose of the meeting was to seek the committee's advice on the guidelines proposed by the Recombinant Advisory Committee. The Advisory Committee to the Director was asked whether, in their judgment, the guidelines balanced scientific responsibility to the public with scientific freedom to pursue new knowledge.

Public responsibility counts heavily in this genetic research area. The scientific community must have the public's confidence that the goals of this profoundly important research accord respect to important ethical, legal, and social values of our society. A key element in achieving and maintaining this public trust is for the scientific community to ensure an openness and candor in its proceedings. Representatives of the international press were invited to the Asilomar conference, and the proceedings received extensive coverage. The meetings of the Director's Advisory Committee and the Recombinant Advisory Committee have also reflected the intent of science to be an open community in considering the conduct of recombinant DNA experiments. Notification of all the meetings was published in the Federal Register and all the meetings were attended and reported by representatives of the press. At the Director's Advisory Committee meeting, there was ample opportunity for comment and an airing of the issues, not only by the committee members but by public witnesses as well. All major points of view were broadly represented.

The guidelines were reviewed in light of the comments and suggestions made by participants at that meeting, as well as the written comments received afterward. As part of that review the Recombinant Advisory Committee was asked to consider at its meeting of April 1-2, 1976, a number of selected issues raised by the commentators. Those issues and the response of the Recombinant Advisory Committee were taken into account in arriving at the final decision on the Guidelines.

The history of the events and discussions leading to the development of the Guidelines are described in greater detail in the "Decision of the Director, NIH," published as a preamble to the Guidelines in the Federal Register, Part II, July 7, 1976 (see Appendix D).

"NIH Guidelines for Recombinant DNA Research." The publication of the NIH Guidelines catalyzed actions designed to bring all use of recombinant DNA techniques under a uniform set of standards. All NIH-supported research was required to follow the Guidelines. Shortly thereafter, the National Science Foundation required this of all its grantees. By 1977 all federally funded research was covered by them. The Pharmaceutical Manufacturers Association, which encompasses the largest body of biomedical scientists having an interest in using recombinant techniques in the absence of Federal support, stated in September 1976 that all of its members would also adhere to the NIH Guidelines (27). As described later, the NIH Guidelines were immediately the object of attention by the worldwide scientific community and formed a basis for the development of similar guidelines in other countries. The NIH Guidelines, first of the national guidelines to be promulgated, were followed by issuance of U.K. guidelines in August 1976 and Canadian guidelines in February 1977. All of these are in general accord with the Asilomar agreement.

The Guidelines were developed because of concern about the possible effects of recombinant DNA research on the environment. Necessarily, therefore, this development included a review of the environmental impact of the research. For example, the objectives of recombinant DNA research were considered and the potential hazards and risks analyzed. Possible alternative approaches to the objectives were thoroughly explored, to maximize safety and minimize potential risks. And a comprehensive review structure to ensure safety has been created. Moreover, in developing the Guidelines, the NIH conscientiously attempted to provide full information to the public and to solicit the public's views.

Section IV-B of the Guidelines defines the roles and responsibilities of institutions in which recombinant DNA methodology is used. The basic mechanism for ensuring local compliance is the institutional biohazards committee. Sections V and VIII of Appendix C of the EIS give additional administrative details concerning these committees, including the requirement that the NIH Office of Recombinant DNA Activities (ORDA) be informed of their establishment and composition. Institutional biohazards committees responsible for recombinant DNA activities (but not necessarily exclusively) have been established in a number of institutions. A listing of these institutions, based on our current information (January 19, 1977), is included in Appendix F to the EIS.

As explained in Appendix C (Section V and Appendix A) of the EIS, the Guidelines (Section IV) require execution of a document entitled "Memorandum of Understanding and Agreement" (MUA) by each investigator and his institution. The MUA must be submitted with all new and renewal applications for grants.

Shortly after the Guidelines were issued, submission of a MUA was required for all ongoing, funded research grants supported by NIH if recombinant DNA technology was utilized. A list of funded projects for which MUAs have been received as of January 1, 1977, is included in Appendix G to the EIS. Readers must realize that the dollar amounts of each listed project cover the entire project, including activities unrelated to recombinant DNA technology and operating costs for laboratory buildings and facilities. It is not possible at present to calculate what portion of funds obligated for each project is related to recombinant DNA activities. In most instances such activities will represent only one of many procedures used, and will constitute a relatively small portion of the total costs.

C. Description of Issues Raised by Recombinant DNA Research

Research is, by definition, investigation of the unknown. The results of research, whether beneficial, neutral, detrimental, or some combination of these, cannot be fully predicted ahead of time. The following discussions are assessments based on present knowledge and collective technical judgments. Unexpected benefits and unexpected hazards are possible.

1. Possible Hazardous Situations

The stable insertion of DNA derived from a different species into a cell or virus (and thus the progeny thereof) may change certain properties of the host. The changes may be advantageous, detrimental, or neutral with regard to (a) the survival of the recipient species, (b) other forms of life that come in contact with the recipient, and (c) aspects of the nonliving environment. Current knowledge does not permit accurate assessment of whether such changes will be advantageous, detrimental, or neutral, and to what degree, when considering a particular recombinant DNA experiment. A major part of this uncertainty is derived from the fact that mere insertion of foreign genes does not automatically affect the host. The genes must be "expressed"--that is, must cause a new protein to be produced--or must otherwise alter function. At present it is only possible to speculate on ways in which the presence of recombinant DNA in a cell might change the cell's properties.

It should be emphasized that there is no known instance in which a hazardous agent has been created by recombinant DNA technology. The following discussion considers ways in which hazardous agents might be produced. In principle, the analysis is applicable to animals, including humans, and plants, when potential effects on complex organisms are described.

a. The effect of foreign DNA on the survival of recipient species (host cell, plasmids, or viruses)

The effect of foreign DNA on the survival of recipient species is important to the discussion of possible hazards of recombinant DNA experiments. A recipient species may acquire a potential for harmful effects as a result of the foreign DNA, but the possibility of the occurrence of the harmful effects will depend on the survival of the altered recipient and its ability to multiply. If acquisition of foreign DNA increases the probability of survival and multiplication, the possibility of harmful effects will increase. Similarly, if acquisition of foreign DNA decreases the probability of survival or multiplication, the possibility of harmful effects will decrease. It is important to recognize, in evaluating the potential for harmful effects, that significant infections of animals and plants by bacteria or viruses usually require contact with far more than a single organism. The critical number of infectious agents will vary, depending on both the agent and the recipient.

Survival of Host Cell. There are multiple indications that both host bacteria and plasmid or virus vectors containing inserted foreign DNA are less likely to survive and multiply than are the original organisms, except for the very unusual instances where the foreign DNA supplies some function, such as antibiotic resistance, that favors the organism in a particular, non-natural environment.* Natural selection results in the survival of only well-balanced and efficient organisms; unneeded genetic material tends to be lost. Essential functions are carefully controlled and are switched on and off as needed. The activity of a particular gene product depends upon, and in turn influences, many other functions of a cell. It is unlikely that uncontrolled, nonessential properties such as might be introduced by foreign genes would result in any advantage to the survival and multiplication of an otherwise well-balanced organism. Similarly, it is unlikely that elimination of a normal gene product caused by insertion of a foreign DNA sequence would be advantageous. More likely than not, any new properties derived from insertion of foreign DNA would confer some relative disability to the recipient organism. Therefore, it is probable that bacterial cells, plasmids, or viruses containing inserted foreign DNA would multiply more slowly in nature than the same cells or vectors without foreign DNA; and in a natural competitive environment, those organisms containing recombinant DNA would generally be expected to disappear. For bacterial hosts, the rate of disappearance would depend on the rate of growth relative to that of competing bacteria. The following calculation demonstrates this point.

*See reference 10 for a general discussion of the analysis in this paragraph.

> Assume that a new organism constitutes 90 percent of a population, but grows 10 percent less rapidly than its natural counterpart. The new organism will drop from a concentration of 90 percent to a concentration of 0.0001 percent (1 part in 1,000,000) in 207 generations. If the generation time of the natural organism is one hour, this decline in concentration will occur in about 8-1/2 days.

Although unlikely, there is a chance that a bacterial host of recombinant DNA will grow more rapidly than if it were lacking the foreign DNA, especially if the cells encounter new environments in which the new gene provides some adaptive advantage. (The calculation given above can also be applied.) A relevant example of such a situation can be found in the rapid and widespread increase in the resistance of bacteria to clinically important antibiotics during the last 20 years. It is well known that such resistance is genetically determined and occurs by natural recombination, and genes specifying resistance have been described (11). Furthermore, it is well known that such genes may be transferred, by natural DNA recombination, from one species of microorganism to another (11). In this instance, the foreign DNA may significantly enhance the survival rate of a bacterial recipient whose environment contains the antibiotic in question, while cells sensitive to the drug will be destroyed. Thus, increased use of antibiotics to cure or prevent infectious disease has been accompanied by increased prevalence of organisms resistant to these antibiotics.

The ability of recipient bacterial host cells to survive and multiply might also be enhanced by acquisition and expression of a foreign gene conferring the ability to metabolize a particular nutrient. In an environmental niche containing the nutrient, such a recombinant might compete successfully against organisms native to the niche. Thus, not only an important nutrient there might be destroyed, but any beneficial functions performed by native organisms could be lost upon the successful establishment of the recombinant in the niche.

These two examples serve to illustrate some of the complexities involved in determining whether the insertion of a given fragment of foreign DNA will be advantageous or disadvantageous to the recipient organism: the nature of the inserted genes, the nature of the environment, and the relation between the two must be considered. However, this analysis is necessarily simplistic. In the absence of the highly specific relationships that are, for example, apparent in the case of antibiotic resistance, very little is understood about how the totality of the genetic make-up of a given organism or species contributes to its competitive advantage even in a defined ecological niche. Modern evolutionary theory does not provide useful frameworks for analysis. There are in fact current major controversies concerning the role of natural mutations in evolution, and the same questions are relevant to the issues raised by recombinant DNA research.

Survival of Vectors. Because potentially useful vectors such as plasmids and viruses may be transferred from cell to cell, independent of the growth and survival of the initial recipient, it is also necessary to consider survival of the vectors. Plasmids and viruses occur widely in nature. Any particular plasmid or virus will normally multiply only within a limited number of species. Thus, for example, viruses that infect particular bacteria neither multiply nor cause disease in the cells of other bacterial species or complex organisms. In many instances, they do not even enter the cells of any organism other than the particular natural host.

Only limited information concerning the effect of foreign DNA insertions on the survival or transferability of plasmid and viral vectors is available. In the case of plasmids, the factors contributing to their maintenance or loss from cells in natural environments, even without insertion of a foreign DNA, are not clearly understood (11). One exception is the selective advantage for maintenance provided by an antibiotic-resistance gene on the plasmid. Also, some plasmids are known to confer on host cells the ability to manufacture substances poisonous to other, closely related cells, thus giving the poison-producers special advantage in a competitive situation. Insertion of a foreign DNA fragment within the DNA sequence coding for the poison has been shown to eliminate production of the poison (4), thus decreasing the likelihood that the cells and their resident recombinant DNA will survive in nature.

Experiments carried out during the last few years have yielded only minimal information on the stability of plasmids containing foreign DNA in E. coli K12 host cells, or on the stability of the foreign fragment itself. For experimental purposes, cells containing recombined plasmids are generally grown under conditions especially designed to increase the stability of the plasmid (called "selective" conditions). For consideration of the loss of the plasmids in natural environments--the important point for matters of safety--the stability of the plasmid or recombined DNA under ordinary, or nonselective, conditions needs to be known. A review of a limited number of unpublished observations indicates that because of the extensive diversity in the nature of the plasmids, and in the nature of the inserted DNA fragment, generalizations as to the rate of loss of the recombined, relative to the original plasmid are impossible. Whether an inserted DNA fragment provides an advantage or a disadvantage to the stability of the plasmid depends not only on the properties of the recombined DNA, but also on the site of insertion of the fragment into the vector, the relation of this site to the gene used to select for recipients of the plasmid, and the conditions of growth.

The ability of a plasmid to be transferred from the original laboratory host to another cell and thereby perpetuate itself is also important. (See Guidelines, Section III-B-1.) In short, certain plasmids are incapable of being transferred except under particular and infrequent conditions. Others transfer more readily. Again, no generalization concerning the effect of a foreign DNA fragment on transferability can be made. Since the ability to be transferred depends on multiple factors (11), it is not likely to be increased by insertion of a single foreign DNA fragment.

Analogous issues as they relate to viral vectors are discussed in some detail in the Guidelines (Section III-B-1 for bacterial viruses and Section III-B-4-a for animal viruses: see also reference 11 of this section.) Two recent papers give evidence that bacteriophage lambda vectors with inserted DNA are less viable than such vectors without inserted DNA (12, 13).

b. The effect of bacteria and viruses containing recombined DNA on other forms of life

The analysis leading to the Guidelines centered on the possibility of deleterious effects, since the concern was the health and safety of living organisms, including humans, and the environment. Agents constructed by recombinant DNA technology could prove hazardous to other forms of life by becoming pathogenic (disease-producing) or toxigenic (toxin-producing), or by becoming more pathogenic or toxigenic than the original agent.

There are two basic mechanisms by which a recipient microorganism might be altered with regard to its pathogenicity or toxicity as a result of a resident recombinant:

(1) The recombinant DNA may result in formation of a protein that has undesirable effects

The case in which bacterial cells are used as carriers of foreign DNA is discussed first. A foreign protein, specified by the foreign DNA, might act after being liberated from the microorganism, or it could function within the microorganism and alter, secondarily, normal microbial cell function in such a way that the cell is rendered harmful to other living things. Either means depends on the expression of the foreign genes; that is, the information in the foreign genes must be used by the recipient bacterium to produce a foreign protein. Examples of proteins that might prove harmful to other organisms are hormones, enzymes, and toxins.

Present evidence suggests that foreign DNA from bacteria of one species, when inserted into bacteria of another species, may be expressed in the recipient, depending on the similarities of the protein synthesis mechanisms in the two organisms (14). For example, if the donor of the foreign DNA produces a toxic substance, then the recipient cell may produce such a substance, provided the gene for the toxic substance is present in the recombinant. The recipient may or may not be more hazardous than the original donor organism, depending on the relative ability of the two organisms to grow and infect an animal or plant species at risk.

The evidence available at present is insufficient to predict whether or not foreign genes derived from a complex organism (animals, plants, yeasts, and fungi) will be expressed in a bacterium in any particular instance (14). It may be that specific manipulations will be required to permit bacteria to express information from a foreign DNA efficiently. Faithful expression of a gene requires accurate functioning of the complex bacterial machinery involved in protein synthesis. At each step, specific signals originating in the foreign gene must be recognized by the bacterial machinery. Evolutionary divergence has resulted in different signals in bacteria and complex organisms (14).

Attempts to translate animal-virus and animal-cell genes into protein, using cell-free systems containing the protein-synthesizing machinery isolated from bacteria such as E. coli, yield some protein-like products. The protein products characterized to date were not faithful to the information in the genes (14).

In a few cases, intact bacteria containing recombined genes from complex organisms have been tested for evidence of expression of the inserted gene (15,16). Accurate expression of the genes has not yet been demonstrated, although it may occur at a low frequency. In some instances, a new protein has been found to replace one encoded by a bacterial gene. This result is expected if a bacterial gene is interrupted by insertion of the new DNA sequence, and does not necessarily indicate expression of the foreign gene.

DNA fragments from yeast have been inserted into a strain of the bacterium E. coli which cannot manufacture the amino acid histidine (16). (Histidine is a component of most proteins and is therefore required for the growth of all organisms.) After insertion, some cells no longer required histidine, the need for which had been overcome by the yeast DNA. This is the first indication that a foreign gene from an organism more complex than bacteria can function in a bacterial cell. (Although yeast is a single-cell organism, it contains an organized nucleus, like cells of higher organisms.)

Analogous issues must be considered for the case in which animal viruses are the carriers of foreign DNA. Many viruses are simply described as DNA molecules enclosed and protected by coats of protein molecules (12,13). The protein coat protects the DNA from environmental effects, thus increasing the ability of the viral DNA to infect a cell. If viral DNAs are recombined with foreign DNAs in such a way that necessary viral genes remain intact, then the recombinant DNA may in turn be able to produce, and be packaged in, the coat of the virus. Inadvertent dispersal of such a viral particle outside of the laboratory might then result in entry of the recombinant DNA into cells of living organisms. The foreign genes may be expressed, resulting in the formation of a protein foreign to the infected cell, or the uncontrolled synthesis of a normal protein. The likelihood of expression of the foreign genes will probably depend on the degree of relatedness between its source and the infected organism (14) as well as its location in the viral DNA used as vector. Currently, few relevant experimental data are available (4).

(2) The recombined DNA may itself cause pathogenic or toxic effects

Foreign DNA inserted in a bacterial gene might so alter the microbial cell's properties that it becomes harmful to other organisms. This might happen, for example, through a change in the growth rate and competitive advantage of the recipient microbial cell, resulting in increased virulence of a mildly pathogenic bacteria. In general, one would expect the inserted DNA to result in a reduced growth rate and a selective disadvantage to the organism, as discussed in IV-C-1-a above. Similar issues arise where animal viruses serve as carriers of foreign DNA.

It is also necessary to consider situations in which DNA molecules themselves may escape from the laboratory or from the experimental host cell and enter cells of living organisms with which they come in contact. Although free DNA molecules are themselves relatively fragile (and the probability that they would survive, in a significant form or for a significant time, in air, water, or any other medium, is considered remote), they can be protected in nature in a variety of ways and be released either into, or close to, a living cell.

When a cell or virus dies, or comes close to or invades the tissue of another living organism, the recombinant DNA may effectively enter a new cell. A hazardous situation similar to that described above might ensue if foreign proteins were manufactured in this "secondary" recipient. The recombinant DNA might survive as an independent cellular component, or it could recombine by natural process with the DNA of the secondary recipient. Various possible deleterious consequences of such a recombination may be considered.

If the secondary recipient is another microorganism, the considerations described in IV-C-1-a apply. If the secondary recipient is one of the cells of an animal or plant, different considerations apply. The possible effects include alterations of normal cellular control mechanisms, synthesis of a foreign protein (such as a hormone), and insertion of genes involved in cancer production (if, for example, the foreign DNA were derived from a cancer-producing virus).

It should be pointed out that the likelihood of inheritable changes in the offspring of complex organisms being caused by such a mechanism is extemely low because of the protection afforded germ-line cells (eggs and sperm) by their location. Thus, it is highly improbable or perhaps impossible for recombined foreign DNA to reach germ-line cells at a time in their life when secondary recombination can occur and affect an offspring. With one-celled organisms, plants, or simple multicellular organisms, the probability of heritable change resulting from secondary recombination is higher.

What is the probability of secondary recombination between prokaryotes and eukaryotes in nature? It is generally held that recombination in nature is more likely if similar or identical sequences of bases (rungs in the DNA ladder) occur in the two recombining DNAs (2). The greater the degree of similar sequences, the more likely is recombination. In general, the more closely two species are related, the more likely it is that similar sequences will be found in their DNAs. Thus, DNA from primates has more DNA sequences in common with human DNA than does DNA from mice, or fish, or plants. Recombination may also occur between DNAs not sharing sequences but at lower frequencies.

It is possible that the capacity for interspecies recombination between distantly related species exists in nature. For example, bacteria in animal intestines are constantly exposed to fragments of animal DNA released from dead intestinal cells. Significant recombination, however, would require the uptake of intact segments of animal DNA and their subsequent incorporation into the bacterial DNA. The frequency of such events, if they occur at all, is unknown.

There are very few available data permitting assessment of the reverse process--namely, the incorporation of bacterial DNA into the cells, or DNA, of more complex organisms. Although there are reports of experiments in which bacterial DNA was inserted into animal and plant species and production of the bacterial protein followed, the process is very inefficient and many investigators have been unable to repeat these experiments (17-19).

There are certain well-documented instances in which the DNAs of different living things become more or less permanently recombined in nature [see Section IV-A of this Environmental Impact Statement]. These instances involve recombination between the DNAs of nonchromosomal genes, such as those of viruses or plasmids, or between the DNAs of viruses or plasmids and chromosomal genes. The former instance, for example, is the mechanism behind the rapid spread of resistance to antibiotics among different bacterial species (see Section IV-C-1 herein and references 11, 20). This spread accompanied the prevalent use of antibiotics in medicine and agriculture. Some viral DNAs recombine into and persist in the chromosomal DNA of cells of receptive organisms (12,13). And some viral DNAs acquire, in stable form, DNA sequences derived from their host cells (21). There is also strong evidence for recombination of the DNA form of RNA animal tumor virus genes with chromosomal genes (22).

2. Expected Benefits of Recombinant DNA Research

Benefits may be divided into two broad categories: an increased understanding of basic biological processes, and practical applications for medicine, agriculture, and industry.

At this time the expected practical applications have not yet been realized and their success remains uncertain. But the ability of recombinant DNA methods to increase understanding of basic biological processes has already been proved. It is important to stress that the most significant results of this work, as with any truly innovative endeavor, are likely to arise in unexpected ways and will almost certainly not follow a predictable path.

a. Increased Understanding of Basic Biological Processes

There are many important fundamental biomedical questions that can be answered or approached by DNA recombinant research (4). In order to advance against inheritable diseases or to understand how man's genetic makeup affects his response to the environment, we need to understand the structure of genes and how they work. The DNA recombinant methodology provides a simple and inexpensive way to prepare large quantities of specific genetic information in pure form. This should permit elucidation of the organization and function of the genetic information in higher organisms. For example, current estimates of the fraction of this information that codes for proteins are simply educated guesses. There are almost no clues about the function of the portions of DNA that do not code for proteins, although these DNA sequences are suspected of being involved in the regulation of gene expression.

The existing state of ignorance is largely attributable to our previous inability to isolate discrete segments of the DNA in a form that permits detailed molecular analysis. Recombinant DNA methodology removes this barrier. Furthermore, ancillary techniques have been developed whereby pure DNA segments that contain particular sequences of interest can be identified and selected. Of particular interest is the isolation of pure DNA segments that contain the genes for the variable and constant portions of the immunoglobin proteins, the substances providing the major resistance of the body to infections or transplants. The analyses of such segments obtained from both germline and somatic cells should be valuable in determining the mechanism of immunologic diversity.

A major problem in understanding the mechanism by which certain viruses cause cancer in animals is how and where the infecting or endogenous viral genomes are integrated into the cell's chromosome (13). This bears on the question of how the expression of the integrated viral genes affects cellular regulation, thus leading to the abnormal growth characteristics of cancer cells. With the recombinant DNA techniques for isolation and purification of specific genes, this research problem is reduced to manageable proportions. It is possible to isolate the desired DNA segment in pure form. Large quantities can be obtained for detailed study by simply extracting a culture of the bacteria carrying the viral DNA segment in a plasmid.

Since the draft EIS was published in September 1976, substantial progress has been made toward realizing the promise of recombinant DNA techniques in the area of basic biological research. The following is an account of what might be done and what has been done (adapted from reference 23).

Much of what is known about the molecular details of gene structure and function has been gathered in studies with simple organisms, principally E. coli and several viruses that infect that bacterium. These studies utilized classical genetic techniques rather than recombinant DNA methods. E. coli is preferred because the organisms are readily grown under controllable laboratory conditions and reproduce rapidly. More importantly, perhaps, their relatively small chromosomes can easily be manipulated by genetic means, and this makes it possible to map the location and arrangement of genes on those chromosomes. This achievement was crucial for understanding how the genes of these organisms are expressed and regulated.

The principal microorganism used with recombinant DNA techniques is a laboratory strain, K-12, of the species, Escherichia coli. The genetic map of E. coli may be represented as a circle: its chromosome is actually a single, circular DNA molecule. About 650 out of an estimated total of 3000-4000 of the organism's genes have been assigned locations on the chromosome. The map provides considerable information--e.g., it shows how some functionally related genes are clustered together and whether the information in a gene is read in a clockwise or counterclockwise direction.

In some regions of the chromosome, the resolution of the map permits us to identify genes that regulate expression of other genes as well as those that code for proteins. One example is a set of genes that govern E. coli's ability to metabolize the milk sugar, lactose. Such models now guide our search for answers about expression and regulation of the genes of higher organisms.

More recently, recombinant DNA experiments have themselves amplified our understanding of bacterial chromosomes and how they work. Recent experiments demonstrated that a segment of DNA can, in living cells, be excised from a chromosome, be turned around, and then reinserted in the opposite orientation. One such inversion controls the type of flagellum (whip-like appendage for locomotion) that the cell makes (24). There are indications that similar mechanisms may operate in higher organisms.

Complexity of Eukaryote DNA. Our extensive knowledge of E. coli's chromosome contrasts sharply with our ignorance about the molecular anatomy of human and other mammalian genomes. As of October 1975, fewer than 150 human genes had been assigned locations on one or another of the 23 chromosome pairs. There could be 5,000-10,000 unknown genes between any two mapped human genes! Although research in this field is flourishing, the acquisition of information about mammalian genomes is slow, costly, and very unlikely to provide the level of resolution achieved with E. coli.

Deciphering the molecular details of E. coli's chromosome (and those of several viral genomes) would not have been accomplished without the ability to isolate specific regions of its DNA in pure form and in large enough quantities for analysis. Scientists confronted with the incredibly complex mixture of DNA fragments obtained from human and other mammalian chromosomes are severely handicapped. But if the complex assortment of DNA fragments could be reassembled in the proper order and the genetic signals deciphered, it would provide a new vision of our genome's structure and would have profound significance for improving human health. Recombinant DNA techniques offer a feasible approach.

With the recombinant DNA method, discrete DNA segments can be isolated in pure form and virtually unlimited quantities from the hopelessly complex mixtures obtained from cells. Molecular cloning provides the means to reconstruct extended regions of chromosomes, and eventually the entire genome, of any organism from the bits and pieces of its DNA. This opens the way for analysis of the detailed molecular structure, e.g. the subunit sequence, of individual genes and extended regions of mammalian chromosomes.

Progress During the Last 3 Years. Recombinant DNA techniques have proved far superior to traditional means for exploring genetic systems. Through use of the new approaches, impressive analyses have already been made of chromosomal segments from simple higher organisms, such as a fruit fly (Drosophila melanogaster), a toad (Xenopus laevis), and sea urchins. Cloned (pure) segments of virtually any chromosome can be obtained.

DNA fragments can be joined to a vector DNA molecule. The vector DNA, a plasmid (or bacterial virus chromosome), and a segment of foreign DNA are joined in the test tube and then introduced into a special strain of E. coli K-12. Conditions can be selected so that only those bacteria or viruses that acquire the recombinant DNA will grow or be detected. Because no more than a single recombinant DNA molecule is taken up by the bacterial cell, all the descendants of that transformed bacterium will contain only one particular foreign DNA segment.

There are a number of different ways to identify cloned mammalian DNA segments and thereby select the clone of interest in a particular experiment. Whatever way they are detected, the size and chemical features of each DNA segment can be analyzed and cataloged.

But can the cloned DNA segments be mapped to chromosomal locations? This has already been accomplished with the DNA of one multicellular organism. Several cloned Drosophila DNA segments have been mapped to specific chromosomal sites. In one instance the data show that the isolated DNA segment occurs at many regions on all four chromosomes. Another cloned DNA segment is found, using the same technique, to occur at only one site on a particular chromosome.

Structural analysis of the chromosomes of simple animals has already transformed our views about the organization of their genes. We know that some genes occur as tandemly repeated, multigene families. And we have learned that some genes contain an insertion of extraneous DNA. The significance of the extraneous insertion is still unclear, but implications for control of gene expression are tantalizing.

We have learned that in different species analogous genes may be organized in different ways and be read off in different directions.

The existence of diverse and sophisticated control mechanisms for turning genes on and off can be inferred. More importantly, the chemical structure of these control elements can now be studied.

Genes corresponding to several important animal proteins have been isolated by recombinant DNA techniques. The DNA corresponding to the gene for globin, the protein part of hemoglobin, has been cloned in an E. coli K-12 system (25). Hemoglobin is the red material that transports oxygen in the blood cells. Also the gene for rat insulin has been cloned (25). These results will now permit detailed study of important features of gene expression and regulation in mammals.

b. Potential Practical Applications for Medicine, Agriculture and Industry.

Certain of the potential applications will be realized only if the reproduction of the recombined foreign DNA in a recipient host cell is followed by expression of the genetic information contained in the DNA in the form of synthesis of proteins. Since the efficient translation of genes of higher organisms (eukaryotes) in bacterial hosts (prokaryotes) has yet to be proved, these potential applications are speculative at this time. Applications that depend on the expression of foreign prokaryotic genes in prokaryotic recipient cells are presently more certain.

(1) Synthesis of Medically Important Proteins and Other Substances

It has been suggested that eukaryote genes coding for medically important substances be attached to bacterial vectors, and that the bacteria then be used to produce large quantities of the desired material. A number of costly or rare substances would be prime candidates for such synthesis:

- human insulin [A future shortage of animal insulin appears likely, and some diabetics are allergic to animal insulins. A first step in the realization of this goal was recently achieved when the gene for rat insulin was successfully cloned in E. coli (25).]
- human growth hormone (presently available only from human cadavers and in short supply)
- clotting factor VIII (for treatment of hemophilia)
- specific antibodies and antigens (for preventing and treating infectious, allergic, and autoimmune disease)

- certain enzymes, such as fibrinolysin and urokinase (promising agents in the treatment of embolism) and lysosomal enzymes. [It has already been shown that a 500-fold increase over the normal amount of one E. coli enzyme can be attained by recombinant DNA techniques (26).]

(2) Endowment of Plants with New Synthetic Capabilities

Whole plants may be generated from a single cell, and thus insertion of recombinant DNA into such cells might make it possible to endow plant species with the capability of--

- improved photosynthetic fixation of carbon dioxide
- nitrogen fixation by presently inept species (thereby reducing the need for chemical fertilizers that are costly or cause pollution--e.g., eutrophication)
- producing a higher quality or quantity of food protein

(3) Some Industrial Applications

A number of industrial processes utilize microorganisms containing enzymes (which are proteins) to produce important chemicals (e.g., steroid hormones, vitamins, or other drugs) or foodstuffs (e.g., cheese). Such processes could be improved through innovations effected by DNA recombinant research. Completely new biosynthetic reactions may thereby become available, permitting the synthesis of large amounts of complex and valuable compounds with ease and at low cost (27).

Some highly speculative applications relate to the area of energy production and neutralization of pollutants--e.g., as in oil spills. Genetic modification through DNA recombination might make it possible to devise microorganisms tailor-made for such important purposes.

3. Long-Range Implications

Another major issue in recombinant DNA research is its long-range implications. The experimental situations treated in the Guidelines are those that appear feasible either currently or in the near future. The experiments primarily involve insertion of recombined DNA into bacteria or into single cells derived from more complex organisms and maintained under special laboratory conditions. It is only in the case of plants that the Guidelines cover experiments involving insertion of DNA into cells capable of developing into complex, multicellular organisms. The Guidelines and the discussions leading to their development have focused on problems of safety.

It is possible that techniques similar to or derived from current recombinant DNA methodology may, in the future, be applicable to the deliberate modification of complex animals, including humans. Such modification might have as its aim correction of an inherited defect in an individual, or alteration of heritable characteristics in the offspring of individuals of a given species. The latter type of alteration has been successfully achieved in agriculture for centuries, by classical breeding techniques. It may be that recombinant DNA methods, should they develop in appropriate ways, will offer new opportunities for specificity and accuracy in animal breeding. It should be noted, however, that the techniques covered by the NIH Guidelines involve the recombining of DNA fragments in the test tube and their insertion into independent single cells only. The techniques do not permit alteration of whole complex organisms.

Application of such methods for the correction of individual genetic defects or the alteration of heritable characteristics in man, should these ever become possible, would pose complex and difficult problems. In addition to questions of concern to individuals, serious societal issues would be involved. Broad discussion in a variety of forums would be indicated.

It would be premature to address these questions in an EIS concerning guidelines for protection against possibly hazardous microorganisms.

Interest in such questions, however, has occasioned activities in both the executive and legislative branches. Concern has been expressed about the implications of certain forms of genetic investigation, ranging from techniques employing recombinant DNA to the broad subject of "genetic engineering." Genetic engineering was discussed at both House and Senate hearings on recombinant DNA legislation. In bills pending in both houses, there are provisions for studies to be undertaken to ensure thorough review of the social, ethical, and legal implications of such techniques to alter the genetic character of man and other forms of life.

4. Possible Deliberate Misuse

A fourth major issue raised by recombinant DNA research is that of deliberate misuse. In the event that recombinant DNA technology proves capable of yielding hazardous agents, such agents might be considered for deliberate perpetration of harm to animals (including humans), plants, or the environment. The possibilities include biological warfare or sabotage. Because it is not known whether recombinant DNA technology can yield such agents, discussion of these problems, such as theft by saboteurs, is hypothetical and difficult. With regard to biological warfare, the use of recombinant DNA for such purposes is prohibited by the Biological Weapons Convention. In a statement to the Conference of the Committee of Disarmament, on August 17, 1976, Ambassador Joseph Martin, Jr., made the following remarks on the subject:

> When advances in science and technology are made, it is natural to ask about their possible use for hostile purposes and whether or not such uses are prohibited or restricted by existing international agreements. In the case of potential use of recombinant DNA molecules for weapons purposes, it is our view that such use clearly falls within the scope of the Convention's prohibition.
>
> This interpretation is based upon the negotiating history as well as the explicit language of the Convention, and we believe that it is shared by the other signatories. I do not believe it is possible to read the Biological Weapons Convention and come to any other conclusion. According to the Preamble, the States Parties are "determined, for the sake of all mankind, to exclude completely the possibility of bacteriological (biological) agents and toxins being used as weapons." The intent of Article I, which begins, "Each State Party to this Convention undertakes never in any circumstances . . . ," is equally forceful and clear. To take a more restricted view would rob the Convention of much of its value and could even lead to States to call into question its scope and continued viability. These were the views of the United States when the Convention was negotiated and ratified. They are still its views today. This is a matter of great importance to my Government and one on which doubt cannot be permitted to exist.

It is noteworthy that, prior to this statement, Dr. David Baltimore had requested an opinion from James L. Malone, General Counsel of the United States Arms Control and Disarmament Agency, on whether the Biological Weapons Convention prohibits production of recombinant DNA molecules for purposes of constructing biological weapons. On July 3, 1975, Mr. Malone replied: "In our opinion the answer is in the affirmative. The use of recombinant DNA molecules for such purposes clearly falls within the scope of the Convention's provisions."

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V.
DESCRIPTION OF THE ACTION

A. General Application of the Guidelines

The Director, National Institutes of Health, has issued Guidelines that govern the conduct of NIH-supported research on recombinant DNA molecules. The Guidelines apply to all NIH-supported research on such molecules--that is, molecules that are made by combining segments of DNA from different organisms in a cell-free system and that can be inserted into some living cell, there to replicate. The objective of the Guidelines is the protection of the laboratory worker, the general public, and the environment from infection by possibly hazardous agents that may result from this research. The complete text of the Guidelines will be found in the Federal Register, Part II, for Wednesday, July 7, 1976. The Guidelines are also included with the present statement as Appendix D. A review of their provisions is given in Appendix E.

The mechanisms by which NIH will implement the application of the Guidelines are outlined in the Guidelines themselves and are specified in greater detail in Appendix C of this Environmental Impact Statement (EIS). Noncompliance with the Guidelines will result in termination of funding of research grants and contracts.

Two broad classes of experiments are described in the Guidelines (Section III). Certain experiments are prohibited. Other experiments are permitted but only under defined conditions designed to limit the spread of the experimental material. There are important distinctions between those classes of experiments that are permitted and those that are prohibited.

Experiments Prohibited by NIH Guidelines. For most recombinant DNA experiments, the possibility that hazardous microorganisms will be produced is purely conjectural. In some possible experiments, however, hazardous organisms would undoubtedly result--for example, when the host cells themselves are hazardous. Such experiments are prohibited. They include those involving microorganisms known to be moderately or highly pathogenic to man, animals, or plants (see 1 and 3 below), and those involving the transfer of antibiotic-resistance traits to microorganisms that do not acquire them naturally, thus limiting the options for treatment of diseases caused by those agents (see 5 below).

In addition, the Guidelines prohibit other experiments that may or may not prove to be risky but for which the nature of the possible hazard can be described and is demonstrably undesirable, such as the formation of recombinant DNAs containing the genes for production of dangerous toxins (see 2 below). Finally, because the escape of substantial numbers of organisms is inherently more likely to yield untoward effects than an accidental release of a small number, should conjectural hazards prove real, the Guidelines prohibit release into the environment of any organisms containing a recombinant DNA molecule (see 4 below) or the carrying out of experiments involving large volumes of recombinant DNA organisms containing harmful material (see 6 below). The following is a more detailed description of the experiments that are prohibited (Section III-A of the Guidelines):

(1) Any experiment in which any portion of the DNA is derived from an organism that is itself a biohazard of greater than low risk as determined by conventional methods of risk-assessment. For this purpose, low-risk organisms correspond to those listed as class 1 or 2 agents by the Center for Disease Control, or as low-risk oncogenic viruses by the National Cancer Institute. The Guidelines further state that the prohibition extends to the use of DNA from any cells known to be infected by agents of greater than low risk.

(2) The deliberate formation of recombinant DNAs containing genes for the biosynthesis of potent toxins. This prohibition precludes the use of DNA from organisms known to produce potent toxins, such as certain bacteria (for example, those producing botulism or diphtheria toxin) and certain insects, amphibians, and snakes (those known to produce potent venoms) unless purified DNA known to be free of the genes for the toxins and venoms is used.

(3) The deliberate creation of recombinant DNAs involving the DNA of plant pathogens, if the recombinants are likely to increase the virulence and host range of the pathogen.

(4) The deliberate release into the environment of any organism containing a recombinant DNA molecule.

(5) The transfer, by recombinant DNA techniques, of genes for drug-resistance traits to microorganisms that are not known to acquire those genes naturally, if such acquisition could compromise the use of the drug to control disease agents in human or veterinary medicine or agriculture. It should be recognized that a large number of microorganisms do acquire such traits in nature, by intra- and interspecies recombination. Therefore, experiments involving, for example, the transfer of a large number of drug resistance traits into E. coli are not prohibited, since E. coli is known to acquire such traits in nature.

(6) Experiments involving more than 10 liters of culture fluid with recombinant DNAs known to make harmful products are prohibited. This is because the probability of escape from containment barriers, especially in the case of accident, increases with increasing scale.

The list of prohibited acts and experiments reflects the assumption, implicit throughout the Guidelines, that recombinant organisms may present greater possible dangers than are present in the most hazardous component used to construct the recombinant DNA.

As noted in Appendix A, the Center for Disease Control's "Classification of Etiologic Agents on the Basis of Hazard" (fourth edition, July 1974) lists the following Classes of etiologic agents: (1) agents of no or minimal hazards under ordinary conditions of handling, (2) agents of ordinary potential hazard, (3) agents involving special hazard, (4) agents that require the most stringent conditions for their containment because they are extremely hazardous to laboratory personnel or may cause serious epidemic disease, and (5) foreign animal pathogens that are excluded from the United States by law or whose entry is restricted by USDA administrative policy.

Cloning of recombinant DNAs derived from organisms in Classes 3, 4, and 5 are prohibited. Prokaryotic hosts other than E. coli strain K-12, to be used for recombinant DNA experiments, must conform to the definition of Class 1 agents. The CDC classification consists primarily of agents etiologic in man. The use in the Guidelines of the CDC classification is based on the recognition that the laboratory worker conducting recombinant DNA research has the highest potential for exposure, and is the most likely route for transmission of microorganisms to other living things.

Special guidance is also provided for strictly animal and plant pathogens. The Guidelines prohibit the use of animal pathogens excluded from the United States by law and USDA administrative policy, and plant pathogens where experiments may increase their virulence and host range. In addition, general guidance for the use of animal and plant host-vector systems is provided.

Experiments Permitted by NIH Guidelines. The permissible experiments include all those for which the risk of producing hazardous agents is thought to be exceptionally low. Further, experiments are only permissible provided that the information to be obtained, or the practical benefits anticipated, cannot be obtained by conventional methods. For the permitted experiments, the safeguards provided by the Guidelines are at least as stringent as those generally used to handle the most hazardous parent of the recombinant.

For permissible experiments the Guidelines describe (1) safeguards that protect the laboratory worker, the general public, and the environment, (2) the criteria for assessing the possible dangers from experiments involving recombinant DNA molecules, (3) the criteria for matching the assessed possible dangers of individual experiments with the appropriate safeguards, and (4) the roles and responsibilities of principal investigators, their institutions, and NIH for ensuring the implementation of the requirements specified in the Guidelines. The emphasis on protection of laboratory workers from infection reflects that those are the persons at the greatest risk of infection and are therefore the most likely route of escape of possibly hazardous agents from the laboratory.

The physical safeguards have been grouped into four levels providing increasing capability for containment. The four levels approximate those recommended by the Center for Disease Control for controlling known infectious agents that have been determined to be of (1) no or minimal, (2) ordinary, (3) special, or (4) extreme hazard to man and other living things. These correspond to the terms minimal, low, moderate, and high risk, respectively, as used in the NIH Guidelines. The safeguards include usual and special microbiological safety practices, primary physical barriers that isolate the experiment from the laboratory worker, and facility installations that either eliminate or markedly reduce the potential for accidental dissemination of recombinant DNA molecules into the environment. The four levels, designated P1 to P4, provide increasing protection against contact with or accidental release of microorganisms containing recombinant DNA molecules.

B. Physical Containment Requirements

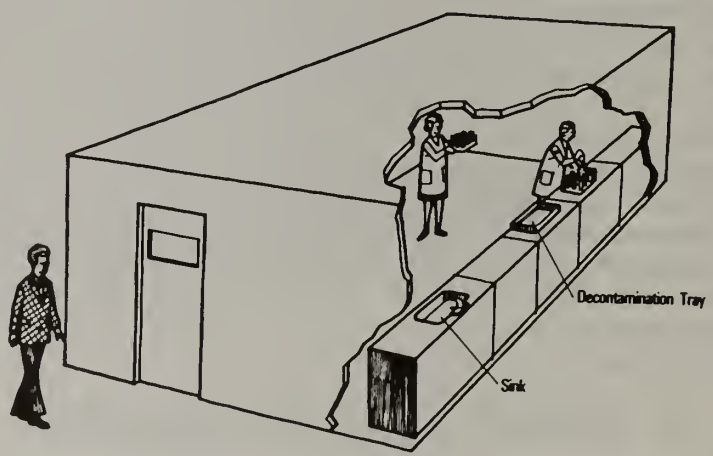
The safeguards in the Guidelines require the use of procedures and physical containment systems to protect laboratory workers and the environment from exposure to potentially harmful organisms. The requirements include procedures and equipment in which work is to be done and special laboratory room and building features, as well as appropriate training of workers. The systems are grouped into four levels of containment--P1, P2, P3, and P4--each providing a level of protection greater than the one preceding it. The level of containment that must be provided by a laboratory in which an experiment is to be done is based on an assessment of the degree of hazard involved.

The following description of the physical containment levels is presented to outline these requirements. A complete description may be found in the Guidelines (Appendix D). Please note that Appendix D to the Guidelines (see Appendix D to this EIS) provides detailed supplementary information on physical containment. Among many other items are specifics on care and use of laboratory animals, requirements for an insect and rodent control program at all containment levels, and guidance on decontamination and disposal of liquid and solid waste.

P1 Level (Minimal). At the P1 level, physical containment is provided by standard microbiological practices. These include antiseptic techniques, the daily decontamination of work surfaces, the decontamination of contaminated liquid and solid wastes, the use of safe pipetting procedures, and the observance of appropriate worker hygienic procedures.

A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P1 level is shown in Figure V-1. Such a laboratory possesses no special engineering design features. Special containment equipment is neither required nor generally available, and the work is usually conducted on open bench tops. The laboratory is not separated from the general traffic patterns of the building, and public access may be permitted.

Figure V-1



P1 Laboratory

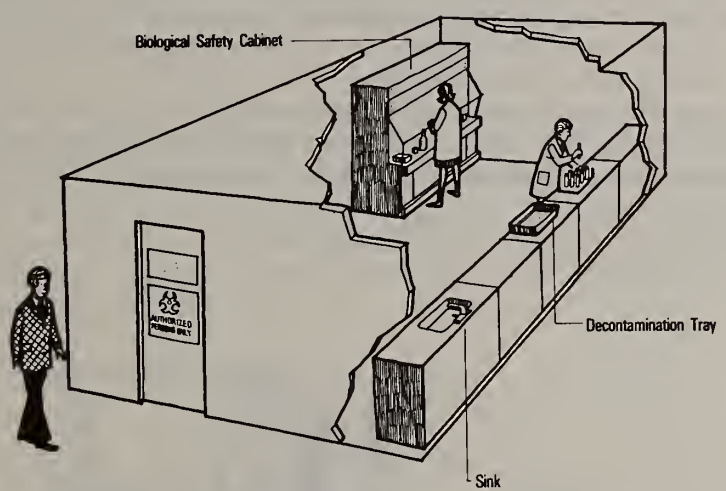
Protective measures equivalent to those described for the P1 level of physical containment are used to control infectious organisms, such as non-pathogenic strains of E. coli, which cause no or minimal hazard under ordinary conditions of handling. This minimal level of containment has also been applied to most studies involving tissue cultures from man, other primates, and lower mammals. Until the 1950s, most organisms that cause disease in man were isolated and studied under conditions equivalent to those described for the P1 level.

P2 Level (Low). Physical containment at the P2 level is provided by a combination of laboratory practices and containment equipment. In addition to those practices required at the P1 level, mechanical pipetting aids are required; eating, drinking, smoking, and storage of food are not permitted in the laboratory; and laboratory garments are required. Containment equipment is required to isolate operations that produce a considerable aerosol.

The principal item of containment equipment is the biological safety cabinet. This is an open-front cabinet, with an inward airflow from the laboratory room of at least 75 linear feet per minute. The cabinet serves primarily to protect the operator from aerosols that may be created by the activities performed within the cabinet; it also serves to prevent the release of airborne contaminants to the environment. The air which is exhausted from these cabinets is either filtered by high-efficiency particulate air filters or incinerated. The high-efficiency particulate air filter is capable of removing more than 99.997 percent of all microorganisms that may be in the cabinet exhaust air.

A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P2 level (see Figure V-2) is identical in construction and design to the P1 laboratory. Access to the P2 laboratory is limited when experiments requiring P2-level physical containment are being conducted.

Figure V-2



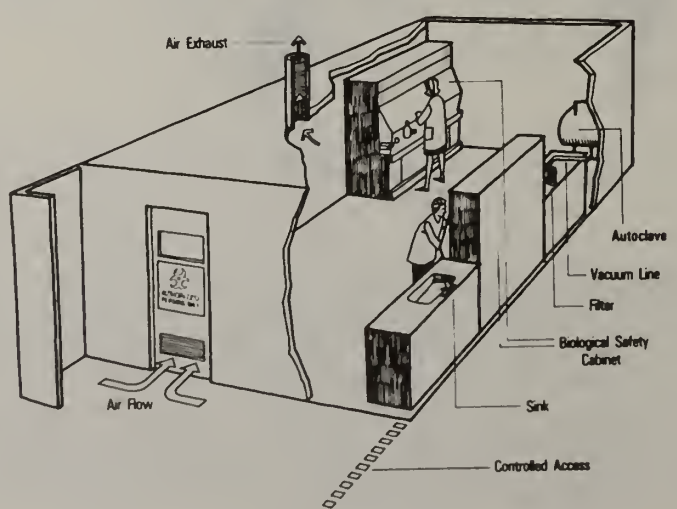
P2 Laboratory

Physical containment procedures required for P2-level work are generally applied as safeguards in the conduct of research with organisms that cause most bacterial and viral diseases of man. For example, these safeguards are recommended by the Center for Disease Control for the containment of bacteria that cause anthrax, diphtheria, pneumonia, gonorrhea, salmonellosis, strep throat, syphilis, and cholera. They are also recommended to contain viruses that cause influenza, measles, mumps, and polio.

P3 Level (Moderate). At the P3 level, even more stringent laboratory practices are required in addition to those mentioned above. For example, long-sleeve solid-front or wrap-around surgical gowns are required to be worn by persons in the laboratory; protective gloves are worn when handling the research materials; hands are washed immediately following the removal of gloves; only persons whose entry into the laboratory is required on the basis of program or support needs can be authorized to enter the laboratory; and vacuum services are protected by filters and disinfectant traps. In addition, all operations that may produce aerosols must be confined to containment equipment.

As shown in Figure V-3, a laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P3 level has special engineering design features and physical containment equipment. The laboratory is separated from areas that are open to the general public. Separation is generally achieved by controlled access corridors and double-door facilities--air locks, locker rooms, etc.--not available for use by the general public. Access to the laboratory is controlled. Biological safety cabinets are available within the controlled laboratory area. An autoclave is available within the building and preferably within the controlled laboratory area. The surfaces of walls, floors, bench tops, and ceilings are easily cleanable to facilitate housekeeping and space decontamination. The laboratory ventilation system is balanced to provide for an inflow of supply air from the access corridor into the laboratory. No work in open vessels is conducted on the open bench; all such procedures are confined to biological safety cabinets.

Figure V-3



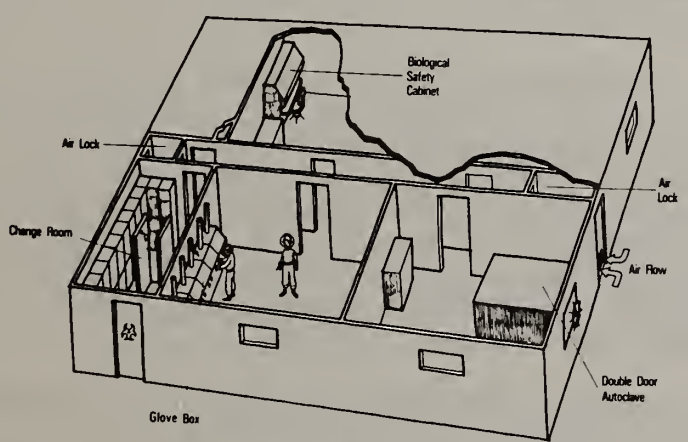
P3 Laboratory

Safeguards provided by P3 physical containment have been used most extensively in the last two decades to ensure the safe conduct of research with organisms that cause serious disease in man. Bacteria that cause brucellosis, tuberculosis, and plague have been successfully contained, as have viral and rickettsial agents that cause rabies, lymphocytic choreomeningitis, Q fever, and typhus fever. These procedures are also appropriate for the containment of research activities using smallpox virus and yellow fever virus when experimental animals are not involved.

P4 Level (High). Physical containment providing the greatest safeguards for reducing the potential for accidental release of microorganisms are used at the P4 level. All research operations involving recombinant DNA materials are confined to Class III biological safety cabinets, which are gas-tight physical enclosures. Operations are performed through gloves attached to the cabinet. The cabinets are maintained under negative air pressure, and the exhaust air from the cabinets is either filtered through two sets of high-efficiency particulate air filters or is filtered once and incinerated. Under ordinary circumstances of operation, they provide an impenetrable barrier between the inside of the cabinet and the laboratory environment.

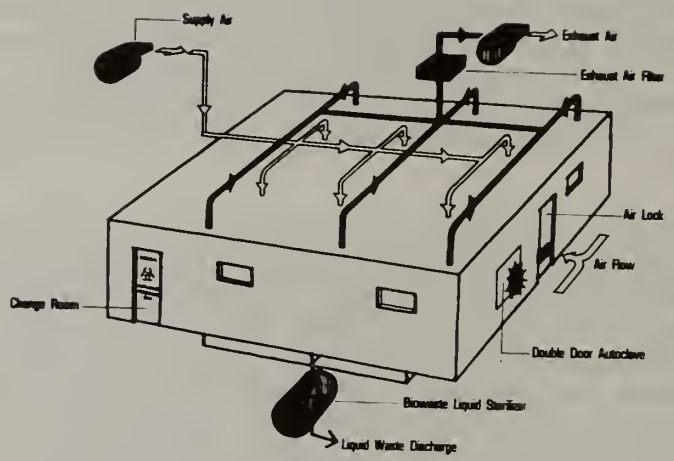
The Class III biological safety cabinets are located in a maximum security facility providing secondary safeguards designed to further reduce the potential for escape of microorganisms to the environment. These features are shown in figures V-4 and V-5. The facility is either a separate building or a controlled area, within a building, that is completely isolated from all other areas of the building. Access to the facility is under strict control.

Figure V-4



P4 Laboratory

Figure V-5



P4 Laboratory Suite
Secondary Barriers

The secondary safeguards of the facility include: (1) monolithic walls, floors, and ceilings in which all penetrations, such as for air ducts, electrical conduits, and utility pipes, are sealed to ensure the physical isolation of the work area and to facilitate housekeeping and space decontamination; (2) air locks through which supplies and materials can be brought safely into the facility; (3) contiguous clothing-change and shower rooms through which personnel enter the facility and exit from it; (4) double-door autoclaves to sterilize and safely remove wastes and other materials from the facility; (5) a biowaste treatment system to sterilize liquid effluents; (6) a separate ventilation system that maintains negative air pressures and directional air flow within the facility; and (7) a treatment system to decontaminate exhaust air before it is dispersed to the atmosphere.

A measure of the degree of safety afforded by P4 containment is the fact that, in the past, such precautions were only applied in work with viruses that are extremely hazardous to laboratory personnel or that may cause serious epidemic disease. For example, these safeguards are recommended by the Center for Disease Control for work with the viruses of hemorrhagic fever, encephalitis, Lassa fever, and Marburg disease. Similarly, such maximum containment has been used in the study of the airborne transmission of pathogens, which involves the generation of highly concentrated aerosols.

Further Information on Physical Containment. An extensive discussion of physical containment is given in Section VII-A-1 and -2. Further, Appendix J (Section V-E) contains information on current NIH activities in biohazard assessment, development of safer host-vector systems, and training.

C. Biological Containment Requirements

Additional safeguards are provided by the use of host cells and vectors with demonstrably limited ability to survive in other than specially designed laboratory environments. This concept is called "biological containment" in the Guidelines.

Confusion can arise because "biological containment" does not, in fact, contain the organisms in the sense that their dispersion from the laboratory is controlled. Rather, the term is used to describe organisms that, were they to escape the laboratory, would have only a slight probability of growing in natural environments. The accidental release of a relatively small number of such organisms should not lead to an amplification of their number, for the original organisms are not likely to survive, and the probability of resulting hazards is thereby diminished.

E. coli K-12 host-vector systems. At present, the system of choice for many experiments is the common laboratory bacterium, E. coli strain K-12, and independent genetic elements (plasmids and bacteriophages) known to reside or replicate in this strain.

The nature and manner of achieving biological containment with this system is described in the Guidelines. E. coli K-12 appears to be a harmless microorganism. It does not usually establish itself in the normal bowel or multiply significantly in the alimentary tract. These facts suggest that accidental ingestion of a small number of bacteria by a laboratory worker would not result in their extensive spread outside the laboratory. The normal situation may be altered when people are either taking antibiotics or have certain abnormal digestive conditions, and it is recommended that such individuals refrain from work for the duration of the abnormal situation.

While E. coli K-12 does not establish itself as a growing strain in normal bowels, it does remain alive during its passage through the tract. Therefore, transfer of plasmid or bacteriophage vectors containing foreign DNA from the original E. coli K-12 host to bacteria resident in the intestines or bacteria encountered after excretion must be considered. For any given host-plasmid combination used in a recombinant DNA experiment, it will be necessary to assess the possibilities for transfer of the recombinant DNA in order to evaluate the degree of biological containment. The available data suggest that the probability of transfer can be quite low, depending on the particular plasmid used and on the viability of the host cell in natural environments. Hence, host-vector systems made up of E. coli K-12 and such plasmids appear to have very limited ability to spread recombinant DNA molecules. (These systems are discussed in detail in Section VI-C.)

Analogous considerations apply when bacteriophages are to be used as vectors for foreign DNA. Bacteriophage vectors could be spread either as mature bacteriophage, or in cells carrying the phage DNA inserted into its own chromosome or as a plasmid. The widely studied bacteriophage lambda might best be used to illustrate relevant considerations, since it is often employed in recombinant DNA experiments.

Considering first its escape as a phage particle, lambda is sensitive to the acidity of the stomach and is likely to be destroyed there. Normal intestinal strains of E. coli are usually not susceptible to infection by lambda, and in fact, susceptible strains are rare in nature. Further, in at least one case, ingestion of 10^{11} (100,000,000,000) lambda particles yielded no detectable lambda in resulting feces. Lambda is also readily destroyed by drying in air. Dissemination of lambda recombinants can be minimized by use of mutant varieties of lambda which lack genes necessary for insertion into the host chromosome: with such phage the frequency of integration into the host chromosome is reduced to 10^{-5} or 10^{-6} (1 in 100,000 to 1,000,000). Finally, conversion of lambda DNA to a stable plasmid is also a relatively unlikely event, occurring at a frequency of about 10^{-6}.

EK1 containment. Considering, then, the properties of E. coli K-12, as well as those of the existing plasmid and bacteriophage vectors, the Guidelines conclude that, in the use of such host-vector systems, recombinant DNAs are highly unlikely to be spread by the ingestion or dissemination of the few hundred or thousand bacteria that might be involved in laboratory accidents, given standard microbiological practice. Therefore, these existing systems, and analogous combinations of E. coli K-12 with other vectors and bacteriophages, are judged to offer a moderate level of biological containment and are defined as EK1, the lowest level of biological containment for experiments with E. coli systems. Other prokaryote host-vector systems need to be evaluated using the same general principles as those applied to the E. coli K-12 situation.

EK2 containment. Increasing numbers after the designation "EK" specify increasing levels of biological containment for E. coli systems. The next level is called "EK2." Its host-vector combinations must be demonstrated by suitable laboratory tests to provide a high level of biological containment. Such combinations are obtained by classical genetic modification (not recombinant DNA) of E. coli K-12 host cells, the relevant plasmids and bacteriophages, or both. More specifically, the Guidelines state that in order to qualify as EK2 the modified system composed of derivatives of E. coli K-12 and a particular vector should not permit survival of a genetic marker carried on the vector in other than specially designed laboratory environments at a frequency greater than 10^{-8} (1 in 100,000,000). Various examples of the types of necessary modifications are suggested in the Guidelines.

EK3 containment; other biological information. One additional level of contained E. coli host-vector systems is defined in the Guidelines and is called EK3. EK3 systems are EK2 systems for which the specified containment properties have been demonstrated not only by microbiological and genetic analysis but by appropriate tests in animals, including humans or primates, and other relevant environments.

EK2 and EK3 host-vector systems must be certified by the Director of NIH after evaluation and recommendation by the Recombinant Advisory Committee. Detailed data on the relevant properties of the system must be submitted for consideration by the Committee. Detailed statements of the criteria that must be met for EK2 certification are included in Appendix H (I and II). Appendix H-III contains a list of already certified EK2 host-vector systems.

As yet no EK3 systems have been submitted for certification. A Workshop on Design of Tests for EK3 Host-Phage-Vector Systems was held at NIH on July 19, 1977. A forthcoming report will be available from the NIH Office of Recombinant DNA Activities.

In the case of a vector derived from an animal virus, the virus itself must be a low-risk agent (as classified by CDC or the National Cancer Institute), and a strain of the virus that is defective in infection must serve as the source of the vector DNA.

D. Combination of Physical and Biological Containment in Practice

Permissible experiments are assigned required levels of both physical and biological containment. The greatest level of safety can be obtained by the complementary use of both. It is precisely because neither form of containment alone is infallible that the complementary use of the two, for every experiment, significantly decreases the likelihood that potentially hazardous organisms will escape and cause significant harm.

Criteria for Selection of Safeguards. The selection of containment (safeguard) levels is dependent on the assessed possible dangers of the experiment. The Guidelines provide standards for evaluating the conceivable dangers of particular experiments involving recombinant DNA molecules. In the absence of evidence of any hazard actually occurring, these standards are based on relevant current knowledge.

The criteria for assessing the possibility for danger are:

(1) the degree to which the DNA segment has been purified away from other genes and shown to be free of harmful characteristics,

(2) the biohazard associated with the DNA of the cell or microorganism that serves as the DNA source (e.g., genes for toxin production),

(3) the biohazard associated with the vector that serves to transmit the source DNA to a recipient host cell,

(4) the ability of the vector to survive in natural environments or habitats,

(5) the kinds and number of different organisms that are susceptible to infection by the vector or recipient,

(6) the biohazard of the recipient host cell that serves to replicate the recombinant DNA molecule,

(7) the ability of the recipient cell to survive in natural environments or habitats,

(8) the ability of the recipient cell to transmit the recombinant DNA molecule to other cells capable of surviving in natural environments or habitats,

(9) the potential of the recipient cell to obtain the source DNA by natural means, and

(10) the evolutionary relatedness of the DNA source to humans. The potential dangers are considered to increase as the organism providing the source DNA approaches humans phylogenetically. Thus, source DNA from primate cells is considered to have greater potential danger than source DNA from lower eukaryotes.

In general, the Guidelines assume that the potential hazards of organisms containing recombinant DNA are greater than those associated with their most hazardous components.

The risk-assessment standards are specified in detail for one prokaryote host-vector system--namely, the E. coli K-12 system. The Guidelines are organized, for the E. coli systems, according to the source and nature of the foreign DNA. For instance, a sample of "foreign" DNA containing essentially all the genetic information of an organism can be isolated, fragmented, and recombined. If the experiment involves such a mixture of DNA fragments, it is referred to as a "shotgun experiment." Such experiments are assumed to be of relatively high potential hazard (Criterion 1 above) because of the greater likelihood that unknown, and therefore possibly hazardous, genes may be introduced into a recipient cell, compared with experiments utilizing a single, highly purified fragment of DNA.

Purified fragments containing mainly genes whose properties are known and are not harmful offer less potential hazard than a shotgun experiment and thus require lower containment.

In some instances the foreign DNA will itself be derived from extrachromosomal genetic elements. Such extrachromosomal elements include the DNA of animal viruses, plant viruses, other eukaryote organelles such as mitochondria and chloroplasts, as well as prokaryote plasmids or bacteriophages of the same type used as vectors. Each of these cases is treated separately in the Guidelines. The prokaryote sources are treated differently, depending on whether the source of the "foreign" DNA is an organism that does or does not recombine genetic information with E. coli in nature. If the experiment mimics events known to occur by natural recombinational processes, then no unique hazard can develop from the recombinant DNA experiment (Criterion 9).

Examples of Containment Systems. Several examples of containment systems as combined in practice will be described here. Table I of Appendix E shows the containment required for shotgun experiments when the foreign DNA is a mixture of fragments derived from eukaryotes. The physical and biological containment levels are listed for various possible DNA sources: both types of containment must be used, as they complement each other. For example, DNA from primates requires the most stringent containment, since the estimated potential hazard, either from genes that might function in humans with untoward effects (Criterion 10) or from pathogenic viral DNAs residing in primate tissue (Criterion 2), is judged to be the most serious. Such experiments require either P3 and EK3, or P4 and EK2. These alternatives reflect the premise that the greater the containment afforded by the host-vector system (Criteria 4, 7 and 8), the lower the physical containment required.

Another point of interest is that in two instances--primates and cold-blooded vertebrates--containment requirements are lower if the DNA is isolated from embryonic tissue or germ-line material. This is based on the observation that such material is less likely to be contaminated by pathogenic viruses or other adventitious agents than is adult tissue. Thus, if the foreign DNA is from adult cold-blooded vertebrates, P2 and EK2 are required, but P2 and EK1 can be used if the DNA is from embryonic or germ-line tissue.

In some instances, the Guidelines require more stringent conditions when the source of foreign DNA is known to be pathogenic or toxigenic, or might be infected with a pathogen, or is known to make a harmful product.

Table II of Appendix E summarizes the Guidelines for shotgun experiments when the source of the DNA is a prokaryotic organism. First those prokaryotes that are known to recombine genetic information with E. coli in nature are considered. The containment requirements are low for this group and vary with the pathogenicity of the source of foreign DNA. When this source is a prokaryote that does not naturally recombine genetic material with E. coli, the containment recommendations are high. It is assumed that the more similar the DNAs of donor and host, the greater the probability of expression of foreign DNA. In those cases where the donor exchanges DNA with E. coli in nature, it is unlikely that recombination experiments will create new genetic combinations never tested by nature. When prokaryote donors not known to exchange DNA with E. coli in nature are used, there is a greater potential for new genetic combinations to be formed and expressed.

Characterized clones obtained from shotgun experiments may not be as potentially hazardous as the original mixture of cells. Cloning of the recipient host cell containing the DNA fragment of interest will be one of the normal aims of any recombinant experiment. The Guidelines state that when a clone has been obtained from a shotgun experiment, and has been rigorously characterized, and when there is sufficient evidence that it is free of harmful genes, then experiments involving the clone can be carried out under P1 and EK1 conditions if the foreign DNA was from a species that recombines genes with E. coli in nature, and under P2 and EK1 conditions if it does not. Permission for such downgrading of containment must be obtained from NIH.

Similarly, when the initial recombination involves a purified segment of the foreign chromosomal DNA, and is then not a shotgun experiment, the potential for growth of a hazardous organism will be less, because the likelihood is lower that an unsuspected hazardous gene is present and because the number of clones that must be examined to obtain the desired clone is markedly reduced. The Guidelines define "purified" (or "enriched") as meaning that the desired segment represents at least 99 percent of the total DNA in the preparation, by weight; and further, they require evidence that no harmful genes are present. Under such circumstances the investigator may lower the containment conditions from these recommended for shotgun experiments with DNA of the same source, either by one step in physical containment or by one step in biological containment. Thus, for example, shotgun experiments with DNA from birds require P3 and EK2. A DNA fragment from birds that is free from harmful genes, and purified to 99 percent purity prior to joining to a vector, would require either P2 and EK2 or P3 and EK1.

Eukaryote host-vector systems using defective viral vectors are also described. Many recombinant DNA experiments will involve the use of systems in which the host cells are eukaryote cells grown singly in tissue culture. These host cells are fragile and fastidious, and there is little or no chance that a living cell could escape from a laboratory in the way that an E. coli cell might. Therefore, containment considerations focus on the vectors in these experiments. Useful vectors may include extrachromosomal DNA elements such as organelle DNA, or the DNA of viruses that infect the particular cells of interest. Given the current state of technology, viral DNAs are most likely to be used as vectors in the near future. Animal viruses can escape a laboratory in a viable form, especially if laboratory workers become infected with them.

Two animal viruses are technically feasible for use at present; they are called "SV40" and "polyoma." Both SV40 and polyoma are oncogenic--that is, they cause tumor formation in newborn small laboratory mammals--and both can transform a variety of cells of mammalian origin in tissue culture. They are classified as low-risk oncogenic viruses by the National Cancer Institute, and the viruses themselves must be handled under conditions equivalent to P2. Although there is no evidence that either virus causes tumors in man, SV40 and related viruses do infect human beings and have been isolated in connection with several human disease states. Polyoma, however, does not infect man, and the Guidelines assume that polyoma inherently affords a higher level of biological containment. Therefore, more stringent physical containment is required for SV40 than for polyoma (Criteria 3, 5).

The Guidelines require that any animal virus DNA used for recombination with a foreign DNA must itself be defective--that is, unable to propagate independently.

With these aspects of biological containment in mind, the Guidelines specify the physical containment required for such experiments, as summarized in Table IV of Appendix E. The particular levels of physical containment depend on the source of the foreign DNA, whether defective polyoma or defective SV40 is the chosen vector, and finally on whether virus particles are produced.

The Guidelines also contain recommendations for experiments in which plant cells serve as hosts for recombinant DNA. These cells might be single plant cells grown under laboratory conditions, or seedlings, plant parts, or small whole plants. This is the only instance, in fact, where the Guidelines address the question of recombinant DNA experiments with whole organisms. Directions are given for modification of the specifications for P1, P2, and P3 physical containment in order to provide conditions appropriate for work with plants.

Vectors for use in experiments with plants include plant organelle DNA, such as the DNA of chloroplasts, and DNA of plant viruses of low pathogenicity and restricted host range. These vectors offer moderate levels of biological containment, and the Guidelines specify the required physical containment levels (outlined in Table V of Appendix E). As before, the requirements are organized according to the source of the foreign DNA.

Theoretically, there are a variety of organisms, both prokaryotes and lower eukaryotes, such as fungi and yeast, which will be useful hosts for experiments with recombinant DNAs. Some may offer the special advantage of not infecting humans, animals, or important ecological niches. A variety of technical developments are needed, however, before useful vectors are available for these systems. The growth characteristics of such hosts indicate that containment problems will be like those for E. coli K-12 hosts. The Guidelines urge development of these systems and point out that the biological and physical containment requirements for them can be derived from the detailed recommendations for E. coli K-12 systems.

The diagram on the following page, from an article by Clifford Grobstein in Scientific American, illustrates the combinations of physical and biological containment required by the NIH Guidelines (9).

Excerpted from The Recombinant-DNA Debate by Dr. Clifford Grobstein.
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PHYSICAL CONTAINMENT	BIOLOGICAL CONTAINMENT (FOR *E. COLI* HOST SYSTEMS ONLY)		
	*EK*1	*EK*2	*EK*3
*P*1	DNA from nonpathogenic prokaryotes that naturally exchange genes with *E. coli* Plasmid or bacteriophage DNA from host cells that naturally exchange genes with *E. coli*. (If plasmid or bacteriophage genome contains harmful genes or if DNA segment is less than 99 percent pure and characterized, higher levels of containment are required.)		
*P*2	DNA from embryonic or germ-line cells of cold-blooded vertebrates DNA from other cold-blooded animals and lower eukaryotes (except insects maintained in the laboratory for fewer than 10 generations) DNA from plants (except plants containing known pathogens or producing known toxins) DNA from low-risk pathogenic prokaryotes that naturally exchange genes with *E. coli* Organelle DNA from nonprimate eukaryotes. (For organelle DNA that is less than 99 percent pure higher levels of containment are required.)	DNA from nonembryonic cold-blooded vertebrates DNA from moderate-risk pathogenic prokaryotes that naturally exchange genes with *E. coli* DNA from nonpathogenic prokaryotes that do not naturally exchange genes with *E. coli* DNA from plant viruses Organelle DNA from primates. (For organelle DNA that is less than 99 percent pure higher levels of containment are required.) Plasmid or bacteriophage DNA from host cells that do not naturally exchange genes with *E. coli*. (If there is a risk that recombinant will increase pathogenicity or ecological potential of host, higher levels of containment are required.)	
*P*3	DNA from nonpathogenic prokaryotes that do not naturally exchange genes with *E. coli* DNA from plant viruses Plasmid or bacteriophage DNA from host cells that do not naturally exchange genes with *E. coli*. (If there is a risk that recombinant will increase pathogenicity or ecological potential of host, higher levels of containment are required.)	DNA from embryonic primate-tissue or germ-line cells DNA from other mammalian cells DNA from birds DNA from embryonic, nonembryonic or germ-line vertebrate cells (if vertebrate produces a toxin) DNA from moderate-risk pathogenic prokaryotes that do not naturally exchange genes with *E. coli* DNA from animal viruses (if cloned DNA does not contain harmful genes)	DNA from nonembryonic primate tissue DNA from animal viruses (if cloned DNA contains harmful genes)
*P*4		DNA from nonembryonic primate tissue DNA from animal viruses (if cloned DNA contains harmful genes)	

"SHOTGUN" EXPERIMENTS USING *E. COLI K*-12 OR ITS DERIVATIVES AS THE HOST CELL AND PLASMIDS, BACTERIOPHAGES OR OTHER VIRUSES AS THE CLONING VECTORS

EXPERIMENTS IN WHICH PURE, CHARACTERIZED "FOREIGN" GENES CARRIED BY PLASMIDS, BACTERIOPHAGES OR OTHER VIRUSES ARE CLONED IN *E. COLI K*-12 OR ITS DERIVATIVES

SOME EXAMPLES of the physical and biological containment requirements set forth in the NIH guidelines for research involving recombinant-DNA molecules, issued in June, 1976, are given in this table. The guidelines, which replaced the partial moratorium that limited such research for the preceding two years, are based on "worst case" estimates of the potential risks associated with various classes of recombinant-DNA experiments. Certain experiments are banned, such as those involving DNA from known high-risk pathogens; other experiments, such as those involving DNA from organisms that are known to exchange genes with *E. coli* in nature, require only the safeguards of good laboratory practice (physical-containment level *P*1) and the use of the standard *K*-12 laboratory strain of *E. coli* (biological-containment level *EK*1). Between these extremes the NIH guidelines prescribe appropriate combinations of increasing physical and biological containment for increasing levels of estimated risk. (In this table containment increases from upper left to lower right.)

Thus physical-containment levels *P*2, *P*3 and *P*4 correspond respectively to minimum isolation, moderate isolation and maximum isolation. Biological-containment level *EK*2 refers to the use of new "crippled" strains of *K*-12 incorporating various genetic defects designed to make the cells' survival outside of laboratory conditions essentially impossible. Level *EK*3 is reserved for an *EK*2-level host-vector system that has successfully passed additional field-testing. Because of the very limited availability of *P*4 facilities and because no bacterial host-vector system has yet been certified by the NIH as satisfying the *EK*3 criteria, the recombinant-DNA experiments now in progress in the U.S. with *E. coli* host systems are with a few exceptions limited to those in the unshaded boxes. Experiments with animal-virus host systems (currently only the polyoma and SV40 viruses) require either the *P*3 or the *P*4 level of physical containment. Experiments with plant-virus host systems have special physical-containment requirements that are analogous to the *P*1-to-*P*4 system.

E. Compliance with the Guidelines

The Guidelines also establish an administrative framework for assigning the responsibility for ensuring safety in recombinant DNA research supported by NIH. (See Appendix C of this EIS and Section IV of the Guidelines.) This responsibility is shared among the principal investigators, their institutions, and NIH. The principal investigators have the primary responsibility for hazard assessment and for implementation of appropriate safeguards. The institutions are responsible for ensuring that the principal investigators have the capabilities for meeting the requirements stipulated in the Guidelines. NIH is responsible for securing an independent assessment of the potential dangers of this research and for ensuring that no research is supported unless the Guidelines are followed.

The Guidelines require that the institutions establish biohazards committees to carry out the institutional responsibility, and stipulate the qualifications and expertise of the committee membership. NIH responsibilities are detailed in the Guidelines (Section IV). They are divided among (1) NIH initial review groups, (2) the Recombinant Advisory Committee, and (3) the NIH staff.

Several factors contribute to the expectation that compliance with the Guidelines by NIH grantees, contractors, and intramural scientists will be achieved. First, there is the fact that noncompliance will result in termination of funding. The importance of this threat is enormous. Adequate funding for research efforts is pivotal to independent investigators. Worthwhile scientific achievement is the main relevant currency--the single most important criterion for reputation, for institutional advancement, and for personal satisfaction--in the scientific community, and achievement is totally dependent on funds to purchase equipment and supplies and to support assistants. Because alternative sources of funds are very limited, NIH funding is of primary importance.

Secondly, the Guidelines require that investigators and their institutions share responsibility for compliance. Local biohazards committees must be fully informed of the activities of the laboratories engaged in use of recombinant technology. They must certify the existence and efficacy of the physical containment required, and must judge the training and competence of those conducting the experiments.

There are, in addition, other factors contributing very substantially to compliance. These arise from the nature of the scientific process and community. Achievement of interesting and important scientific results is meaningless unless they are shared with colleagues--by informal communication and finally by publication in the scientific literature. It is partly for this reason that scientists have evolved extensive oral and written outlets for communication. The Guidelines recommend that all publications dealing with recombinant DNA experiments state the containment measures used. And increasingly, scientific articles include such statements.

Furthermore, most laboratories are open institutions. Students and colleagues come and go; visits from colleagues from other institutions are frequent. Publicity regarding any given experiment together with the familiarity of others regarding the laboratory facilities and practices assures that noncompliant experimentation will be disclosed. There will be considerable and constant pressure on investigators to comply. The pressure will come formally from responsible institutional officers, local biohazards committees, and NIH review of both applications and MUAs, as well as informally from colleagues and students.

The extensive compliance with earlier voluntary guidelines and deferrals concerning recombinant DNA research indicates the serious commitment of the scientific community to the goals of the NIH Guidelines.

This discussion is not meant to imply that NIH can predict absolute compliance. For there are problems, as noted by the commentators, in ensuring the adequacy of the "self-policing mechanisms" within the Guidelines. Some individuals may at times be less than vigorous in use of the required procedures, and some may be less well trained than others. It is for these reasons that the requirements in the Guidelines involve many overlapping approaches to containment, each serving to control breaches in the others. It is also for these reasons that the containment requirements have been made stricter than the risk-assessments suggest are necessary.

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VI.
DESCRIPTION OF ALTERNATIVES

The following general classes of action have been considered as alternatives to, or additions to, the issuance of NIH Guidelines for Recombinant DNA Research. The impact of each is described briefly, and reference is made to other portions of this document that have a more complete discussion of the particular impact in question.

A. No Action

This alternative--failure to issue guidelines--would have perpetuated the situation existing prior to June 23, 1976. At that time the only restrictions on recombinant DNA research stemmed from informal voluntary agreement of the research community to abide by guidelines which had been developed at the International Conference on Recombinant DNA Molecules, held at Asilomar, California, in February 1975, and which had been published in scientific journals. The Asilomar guidelines differ in substance from the NIH Guidelines, and are considerably less stringent and less detailed in their requirements for containment of potentially hazardous organisms. For example, experiments that may be carried out with minimal containment according to the specific language of the Asilomar guidelines (e.g., the construction of an E. coli plasmid containing the noncancer-producing DNA segment of SV40) require P3 or P4 according to the NIH Guidelines. In addition, a list of experiments recommended to be deferred in the Asilomar guidelines is expanded in the NIH Guidelines. Furthermore, disregard of the Asilomar guidelines carries no sanctions on investigators, and it might be assumed that the initial high level of voluntary compliance would slowly erode if a more formal and explicit structure were not provided, particularly by the principal supporter of such research in the United States.

As noted previously, the restrictions contained in the NIH Guidelines were intended to minimize the risk of harm to the environment. Further, it should be pointed out that pending bills introduced in the House of Representatives and Senate require compliance with the NIH Guidelines for all who engage in recombinant DNA activities in the Nation. It should also be noted that certain types of experiment now covered in the Guidelines would no longer be subject to regulation in the bills.

B. NIH Prohibition of Funding of All Experiments with Recombinant DNA

NIH could refuse to fund any recombinant DNA experiments. Because NIH funds a large proportion of the Nation's biomedical research effort, a reduction in risks might be achieved by total NIH prohibition. Such research would not cease, however, since it would still be supported by non-NIH funds both in this country and abroad.

At the time of the issuance of the NIH Guidelines, the British government had formally accepted the Ashby report, which concluded that recombinant DNA techniques would continue to be used in Britain. The U.K. Guidelines to implement this appeared in August 1976, two months after the NIH Guidelines were released. As described later, the 16 member nations of the European Science Foundation and Canada, the Soviet Union, and Japan have all declared intentions to continue using the recombinant DNA technology under guidelines that are similar to NIH's. If the hypothetical risks presumed to lie in this research are realized, they are quite unlikely to respect national boundaries or to be contained geographically.

On the other side, if NIH refused to fund any recombinant DNA experiments, its important role in biomedical research makes it very probable that the development of basic knowledge derived from this technology and the possible realization of benefits would be significantly delayed. It is widely anticipated that a variety of research--impacting on health and other areas of human concern--will benefit from recombinant DNA technology (see Section IV-C-2).

American scientists have played a leading role in bringing the potential hazards of recombinant DNA research to the attention of scientists, governments, and international organizations. As a result, there is an effort to adopt safety procedures for the conduct of this research in many countries. Although nations differ in their perceptions of the need to adopt safety measures, and of what the exact measures should be, the NIH Guidelines are being used as an important model. NIH prohibition of the work would undermine American leadership in the establishment of worldwide standards for safety.

It is estimated that there are approximately 150 recombinant DNA projects under way in Europe and perhaps 20 to 25 in Canada, Australia, Japan, and the Soviet Union. All are being conducted under some form of safety practice and procedure.

It should be noted that a number of bills have been introduced into the U.S. Congress in the last year. None called for prohibition of funding of all experiments with recombinant DNA. A number of local communities in the United States have studied the issue; none called for total prohibition.

The issue of recombinant DNA research has been studied by national bodies in many countries throughout the world and by a number of international organizations. In no cases has a total ban of the research been advocated. The United Kingdom and Canada have issued guidelines which differ in detail but are similar conceptually to the NIH Guidelines. Other countries are generally following the U.S. or U.K. guidelines, including Denmark, the Netherlands, France, Federal Republic of Germany, Israel, Sweden, and Switzerland. Endorsement for this position has been given by the European Science Foundation, the European Molecular Biology Organizations, the International Council of Scientific Unions, and the World Health Organization.

The effect of a total ban in the United States would be to deprive humanity of any benefits of the research that would have been done here. These are described in Section IV-C-2. Another statement of the potential benefits is given in the article "Recombinant DNA: Fact and Fiction" by Stanley N. Cohen, in the February 18, 1977, issue of Science (1). An excerpt appears on the following page:

C. Development of Different Guidelines

Another alternative action was the issuance of materially different guidelines. Each of the stipulations in the NIH Guidelines was made after assessment of the possible hazards associated with particular experiments. The available data, however, were limited, and different conclusions could have been reached. Some issues addressed in the preparation of the Guidelines which could have led to different specifications are as follows:

1. Levels of Physical Containment

For those experiments in which the potential risk is entirely hypothetical or at least controversial, the physical containment levels assigned could have been higher or lower. Examples of controversial issues are the recommendations with respect to containment levels for recombinant experiments involving bacterial cells and DNA derived from cold-blooded vertebrates (which some scientist feel should be under higher containment levels), and for experiments involving the use of DNA from animal viruses (which some scientists feel should be under lower containment levels). Indeed guidelines issued by the United Kingdom and by Canada differ from the NIH Guidelines in the exact levels of physical containment assigned to different specific experiments. It is expected that as new information is obtained, the Guidelines will be revised periodically to modify containment levels required.

Excerpted from "Recombinant DNA: Fact and Fiction," Cohen, Stanley N., Science Vol. 195, pp. 654-657, 18 February 1977. Copyright © 1977 by the American Association for the Advancement of Science.

For all but a very few experiments, the risks of recombinant DNA research are speculative. Are the benefits equally speculative or is there some factual basis for expecting that benefits will occur from this technique? I believe that the anticipation of benefits has a substantial basis in fact, and that the benefits fall into two principal categories: (i) advancement of fundamental scientific and medical knowledge, and (ii) possible practical applications.

In the short space of 3½ years, the use of the recombinant DNA technology has already been of major importance in the advancement of fundamental knowledge. We need to understand the structure and function of genes, and this methodology provides a way to isolate large quantities of specific segments of DNA in pure form. For example, recombinant DNA methodology has provided us with much information about the structure of plasmids that cause antibiotic resistance in bacteria, and has given us insights into how these elements propagate themselves, how they evolve, and how their genes are regulated. In the past, our inability to isolate specific genetic regions of the chromosomes of higher organisms has limited our understanding of the genes of complex cells. Now use of recombinant DNA techniques has provided knowledge about how genes are organized into chromosomes and how gene expression is controlled. With such knowledge we can begin to learn how defects in the structure of such genes alter their function.

On a more practical level, recombinant DNA techniques potentially permit the construction of bacterial strains that can produce biologically important substances such as antibodies and hormones. Although the full expression of higher organism DNA that is necessary to accomplish such production has not yet been achieved in bacteria, the steps that need to be taken to reach this goal are defined, and we can reasonably expect that the introduction of appropriate "start" and "stop" control signals into recombinant DNA molecules will enable the expression of animal cell genes. On an even shorter time scale, we can expect recombinant DNA techniques to revolutionize the production of antibiotics, vitamins, and medically and industrially useful chemicals by eliminating the need to grow and process the often exotic bacterial and fungal strains currently used as sources for such agents. We can anticipate the construction of modified antimicrobial agents that are not destroyed by the antibiotic inactivating enzymes responsible for drug resistance in bacteria.

In the area of vaccine production, we can anticipate the construction of specific bacterial strains able to produce desired antigenic products, eliminating the present need for immunization with killed or attenuated specimens of disease-causing viruses.

One practical application of recombinant DNA technology in the area of vaccine production is already close to being realized. An *E. coli* plasmid coding for an enteric toxin fatal to livestock has been taken apart, and the toxin gene has been separated from the remainder of the plasmid. The next step is to cut away a small segment of the toxin-producing gene so that the substance produced by the resulting gene in *E. coli* will not have toxic properties but will be immunologically active in stimulating antibody production.

Other benefits from recombinant DNA research in the areas of food and energy production are more speculative. However, even in these areas there is a scientific basis for expecting that the benefits will someday be realized. The limited availability of fertilizers and the potential hazards associated with excessive use of nitrogen fertilizers now limits the yields of grain and other crops, but agricultural experts suggest that transplantation of the nitrogenase system from the chromosomes of certain bacteria into plants or into other bacteria that live symbiotically with food crop plants may eliminate the need for fertilizers. For many years, scientists have modified the heredity of plants by comparatively primitive techniques. Now there is a means of doing this with greater precision than has been possible previously.

Certain algae are known to produce hydrogen from water, using sunlight as energy. This process potentially can yield a virtually limitless source of pollution-free energy if technical and biochemical problems indigenous to the known hydrogen-producing organisms can be solved. Recombinant DNA techniques offer a possible means of solution to these problems.

It is ironic that some of the most vocal opposition to recombinant DNA research has come from those most concerned about the environment. The ability to manipulate microbial genes offers the promise of more effective utilization of renewable resources for mankind's food and energy needs; the status quo offers the prospect of progressive and continuing devastation of the environment. Yet, some environmentalists have been misled into taking what I believe to be an antienvironmental position on the issue of recombinant DNA.

2. The Recommendation That All Permissible Recombinant DNA Experiments Be Conducted in National Facilities

Advantages to working in national facilities include:

(a) Construction funds could be conserved by building a few regional facilities rather than constructing or renovating space at each institution where this research might be conducted.

(b) Facilities might be more uniformly available to qualified investigators from a variety of institutions.

(c) There could be greater assurance that the facilities meet the specified requirements for physical containment.

(d) Training, supervision, and monitoring could be centralized.

(e) Sites could be placed away from population centers.

The disadvantages of national facilities include:

(a) Many experiments will require only brief access to the facilities on a given day but repeated visits over a lengthy period of time. Investigators will find it inconvenient and often impossible to carry out such "commuter" activities. These difficulties are compounded by the fact that recombinant techniques are often only a small part of the research problem; relevant equipment and materials would have to be transported repeatedly.

(b) There will be problems of access and scheduling related to the limited number of facilities.

(c) Competition for access will likely favor established investigators and established ideas.

(d) The Guidelines and pending regulations are already imposing a fairly severe rate limitation on the exploitation of recombinant technologies. To the degree that this has occurred so far, such conservatism is probably justifiable. There can be no justification, however, for further restriction on the independence and opportunity of scientific inquiry by arbitrary confinement to a few locations for an area of biomedical science whose proved benefits have so overshadowed the hypothetical risks.

This alternative postulates that all experiments involving recombinant DNA molecules, regardless of assessed potential hazard, would be conducted in national facilities. It should be pointed out that many of the experiments covered by the Guidelines are widely perceived as involving no possible danger. For such experiments, there can be no justification for confinement to national laboratories. Consideration should be given to the alternative of limiting to such laboratories all permissible experiments judged to present a chance of producing hazardous agents. (The Guidelines already prohibit all experiments that are demonstrably hazardous.) For the permissible experiments judged to present the greatest conjectural hazard, maximum-containment (P4) laboratories are required. These will certainly be limited in number and can have all the advantages listed above without the concomitant disadvantages.

With regard to experiments presenting lesser degrees of presumed (hypothetical) hazard, many biomedical research facilities are experienced in supporting research involving minimal, low, and moderate biohazardous agents. These existing facilities should not be excluded from supporting recombinant DNA research without good cause. The experience of a hundred years of laboratory investigations of known pathogenic agents gives no evidence of negative impact on the environment. Further, to impose highly restrictive impediments to research, in the absence of a proven hazard, would seem unwarranted.

Nevertheless, some critics of the pattern of safeguards that have evolved--from Asilomar guidelines to NIH guidelines and control apparatus--propose far more stringent measures, such as a highly restricted Federal monopoly on all recombinant DNA experiments. See the letter to Science by Dr. Erwin Chargaff quoted on the following page (29).

3. Experiments Prohibited at This Time

Certain types of experiments are prohibited by the NIH Guidelines. (see Section V of the EIS and Section III-A of the Guidelines). The prohibited experiments include all those that have a definite and demonstrable potential for the production of undesirable organisms. The prohibited experiments also include some about which there is considerable uncertainty as to the potential of the experiments to yield undesirable organisms. These have been included because they might present serious difficulties for humans, animals, plants, or the environment should they prove to be hazardous at all.

Alternative actions would result either in deletions from, or additions to, the list of prohibited experiments. Although it has been argued that available methods can adequately contain even those organisms that might arise from the prohibited experiments, it seems prudent to continue these prohibitions, for much valuable information can be obtained with the experiments that are still permitted. Some of the controversial items are--

Letter by Chargaff, Erwin, Science Vol. 192, pp. 939-940, 4 June 1976.
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Letters

On the Dangers of Genetic Meddling

A bizarre problem is posed by recent attempts to make so-called genetic engineering palatable to the public. Presumably because they were asked to establish "guidelines," the National Institutes of Health have permitted themselves to be dragged into a controversy with which they should not have had anything to do. Perhaps such a request should have been addressed to the Department of Justice. But I doubt that they would have wanted to become involved with second-degree molecular biology.

Although I do not think that a terrorist organization ever asked the Federal Bureau of Investigation to establish guidelines on the proper conduct of bombing experiments, I do not doubt what the answer would have been; namely, that they ought to refrain from doing anything unlawful. This also applies to the case under discussion: no smokescreen, neither P3 nor P4 containment facilities, can absolve an experimenter from having injured a fellow being. I set my hope in the cleaning women and the animal attendants employed in laboratories playing games with "recombinant DNA"; in the law profession, which ought to recognize a golden opportunity for biological malpractice suits; and in the juries that dislike all forms of doctors.

In pursuing my quixotic undertaking—fighting windmills with an M.D. degree—I shall start with the cardinal folly, namely, the choice of *Escherichia coli* as the host. Permit me to quote from a respected textbook of microbiology (*1*): "*E. coli* is referred to as the 'colon bacillus' because it is the predominant facultative species in the large bowel." In fact, we harbor several hundred different varieties of this useful microorganism. It is responsible for few infections but probably for more scientific papers than any other living organism. If our time feels called upon to create new forms of living cells—forms that the world has presumably not seen since its onset—why choose a microbe that has cohabited, more or less happily, with us for a very long time indeed? The answer is that we know so much more about *E. coli* than about anything else, including ourselves. But is this a valid answer? Take your time, study diligently, and you will eventually learn a great deal about organisms that cannot live in men or animals. There is no hurry, there is no hurry whatever.

Here I shall be interrupted by many colleagues who assure me that they cannot wait any longer, that they are in a tremendous hurry to help suffering humanity. Without doubting the purity of their motives, I must say that nobody has, to my knowledge, set out clearly how he plans to go about curing everything from alkaptonuria to Zenker's degeneration, let alone replacing or repairing our genes. But screams and empty promises fill the air. "Don't you want cheap insulin? Would you not like to have cereals get their nitrogen from the air? And how about green man photosynthesizing his nourishment: 10 minutes in the sun for breakfast, 30 minutes for lunch, and 1 hour for dinner?" Well, maybe Yes, maybe No.

If Dr. Frankenstein must go on producing his little biological monsters—and I deny the urgency and even the compulsion—why pick *E. coli* as the womb? This is a field where every experiment is a "shotgun experiment," not only those so designated; and who knows what is really being implanted into the DNA of the plasmids which the bacillus

will continue multiplying to the end of time? And it will eventually get into human beings and animals despite all the precautions of containment. What is inside will be outside. Here I am given the assurance that the work will be done with enfeebled lambda and with modified, defective *E. coli* strains that cannot live in the intestine. But how about the exchange of genetic material in the gut? How can we be sure what would happen once the little beasts escaped from the laboratory? Let me quote once more from the respected textbook (*1*): "Indeed, the possibility cannot be dismissed that genetic recombination in the intestinal tract may even cause harmless enteric bacilli occasionally to become virulent." I am thinking, however, of something much worse than virulence. We are playing with hotter fires.

It is not surprising, but it is regrettable that the groups that entrusted themselves with the formulation of "guidelines," as well as the several advisory committees, consisted exclusively, or almost exclusively, of advocates of this form of genetic experimentation. What seems to have been disregarded completely is that we are dealing here much more with an ethical problem than with one in public health, and that the principal question to be answered is whether we have the right to put an additional fearful load on generations that are not yet born. I use the adjective "additional" in view of the unresolved and equally fearful problem of the disposal of nuclear waste. Our time is cursed with the necessity for feeble men, masquerading as experts, to make enormously far-reaching decisions. Is there anything more far-reaching than the creation of new forms of life?

Recognizing that the National Institutes of Health are not equipped to deal with a dilemma of such import, I can only hope against hope for congressional action. One could, for instance, envision the following steps: (i) a complete prohibition of the use of bacterial hosts that are indigenous to man; (ii) the creation of an authority, truly representative of the population of this country, that would support and license research on less objectionable hosts and procedures; (iii) all forms of "genetic engineering" remaining a federal monopoly; (iv) all research eventually being carried out in one place, such as Fort Detrick. It is clear that a moratorium of some sort will have to precede the erection of legal safeguards.

But beyond all this, there arises a general problem of the greatest significance, namely, the awesome irreversibility of what is being contemplated. You can stop splitting the atom; you can stop visiting the moon; you can stop using aerosols; you may even decide not to kill entire populations by the use of a few bombs. But you cannot recall a new form of life. Once you have constructed a viable *E. coli* cell carrying a plasmid DNA into which a piece of eukaryotic DNA has been spliced, it will survive you and your children and your children's children. An irreversible attack on the biosphere is something so unheard-of, so unthinkable to previous generations, that I could only wish that mine had not been guilty of it. The hybridization of Prometheus with Herostratus is bound to give evil results.

Most of the experimental results published so far in this field are actually quite unconvincing. We understand very little about eukaryotic DNA. The significance of spacer regions, repetitive sequences, and, for that matter, of heterochromatin is not yet fully understood. It appears that the recombination experiments in which a piece of animal DNA is incorporated into the DNA of a microbial plasmid are being performed without a full appreciation of what is going on. Is the position of one gene with respect to its neighbors on the DNA chain accidental or do they control and regulate each other? Can we be sure—to mention one fantastic improbability—that the gene for a given protein hormone, operative only in certain specialized cells, does not become carcinogenic when introduced naked into the intestine? Are we wise in getting ready to mix up what nature has kept apart, namely the genomes of eukaryotic and prokaryotic cells?

The worst is that we shall never know. Bacteria and viruses have always formed a most effective biological underground. The guerilla warfare through which they

act on higher forms of life is only imperfectly understood. By adding to this arsenal freakish forms of life—prokaryotes propagating eukaryotic genes—we shall be throwing a veil of uncertainties over the life of coming generations. Have we the right to counteract, irreversibly, the evolutionary wisdom of millions of years, in order to satisfy the ambition and the curiosity of a few scientists?

This world is given to us on loan. We come and we go; and after a time we leave earth and air and water to others who come after us. My generation, or perhaps the one preceding mine, has been the first to engage, under the leadership of the exact sciences, in a destructive colonial warfare against nature. The future will curse us for it.

ERWIN CHARGAFF

350 Central Park West,
New York 10025

References

1. B. D. Davis, R. Dulbecco, H. N. Eisen, H. S. Ginsberg, W. B. Wood, Jr., *Microbiology* (Harper & Row, New York, ed. 1, 1967), p. 769.

a. The prohibition of experiments involving more than 10 liters of culture fluid containing recombinant DNAs known to make harmful products, without the express approval of the NIH Recombinant Advisory Committee.

It has been suggested that the prohibition on experiments involving more than 10 liters of culture fluid containing recombinant DNAs known to make harmful products be deleted. Controversy over this prohibition relates to the fact that some investigators and laboratories contend that larger volumes of culture fluid can be safely contained by available special procedures and facilities. They further contend that this prohibition will hamper certain potential benefits of recombinant DNA research, such as the large-scale and/or commercial preparation of clinically or industrially useful materials.

b. Use of the bacterium Escherichia coli as a recipient for recombinant DNA molecules.

It has been suggested that the use of the species of bacteria called Escherichia coli as a recipient for recombinant DNA molecules be included in the list of prohibited experiments. There are, in fact, a large number of different subspecies (called strains) of bacteria that are called E. coli. All these strains share certain properties commonly used for the classification of bacterial species, and they are all therefore called Escherichia coli, but they also differ from one another in substantial ways. Because two or more bacterial strains are classified as E. coli does not indicate that they are identical (2, 3). Moreover, some of the strains classified as E. coli are known to be pathogenic and others are not (2, 3). The Guidelines permit the use of only one particular strain of nonpathogenic E. coli, called K-12. There is considerable difference between the ecology and epidemiology of K-12 and those E. coli strains that are pathogenic or commonly inhabit mammalian intestinal tracts.

The laboratory variants of K-12 to be used in recombinant DNA experiments have never been reported to cause disease, even in laboratory workers. K-12 has been grown in very large quantities (up to hundreds of liters containing up to 10^{12} --that is, 1,000,000,000,000-- bacteria per liter at a time) in hundreds of laboratories all over the world over a period of 30 years and under containment conditions lower than those specified as P1 in the Guidelines. Furthermore, K-12 has none of the many properties generally associated with pathogenic bacteria (2, 3, 4, 5, 6, 7, 8, 9). K-12 does not--

- survive and multiply readily in natural environments, and is thus unable to spread from animal to animal or plant to plant;
- multiply readily on body surfaces or intestines and lungs;
- penetrate animal cells;
- spread throughout animal bodies;
- produce a toxin or otherwise alter other living things to cause symptoms associated with disease
- resist normal body defense mechanisms.

Experiments (4, 5, 6, 8) have shown that even after normal humans have ingested up to 10^{10} (10,000,000,000) K-12 cells, only transient multiplications of the bacteria in the intestines can be observed, and that after a time no K-12 can be detected in the feces. Thus, K-12 does not establish itself as a permanent resident of normal human beings.

It might be pointed out that animals, including humans, ingest large numbers of bacteria of many species daily. Most of these do not take up long-term residency. For example, a normal portion of yogurt may contain ten billion cells of the bacteria Lactobacillus vulgaris; in spite of daily consumption, the Lactobacillus quickly disappears from the human bowel. Similar data are available for certain laboratory animals (7, 8).

K-12 can reside in certain abnormal environments, such as the intestines of laboratory-bred germ-free animals (8), and may establish itself in other abnormal conditions such as during antibiotic therapy (10). The Guidelines therefore prohibit investigators from carrying out recombinant DNA experiments when such abnormal conditions exist.

Pathogenicity depends on the complex interrelation between several properties of a given strain and the properties of the infected organism (2). For example, even pathogenic strains of E. coli (and other organisms) may be carried in certain parts of the intestines or in other parts of the body without causing disease. Their ability to cause disease depends on their residence in, and infection of, a very particular locality. Thus, in order to cause disease, they must have both the genetic capability for pathogenicity and a genetically determined ability to establish themselves in a suitable environment.

It is difficult to conceive how K-12, itself nonpathogenic, could become pathogenic as a result of either spontaneous changes or changes brought about by manipulations in recombinant DNA experiments. After many years of laboratory manipulation, K-12 is highly attenuated. Its only known habitat is in biological laboratories. One laboratory which has studied the pathogenesis of E. coli infections for the past 10 years, reports having observed no genetic modification of K-12 which was sufficient to permit the strain to cause overt disease in any animal model studied (9).

Even when genetic determinants known to cause pathogenicity in other E. coli strains were introduced into K-12, no instance of conversion to pathogenicity was detected, either with regard to diarrheal disease or urinary tract infection (8, 9). Certain genetic manipulations did yield K-12 that was better able to multiply within the gastrointestinal tract, but these organisms were usually less robust than the E. coli strains normally found in feces (not K-12).

This result was in contrast to that obtained with fecal E. coli strains (not K-12) which could be converted to pathogenic forms by the same manipulations. These workers concluded that the inadvertent transformation of K-12 into a highly pathogenic form by the acquisition of a single fragment of foreign DNA in a recombinant DNA experiment was highly unlikely (9).

The possiblity that K-12 could become pathogenic is discussed in detail in a paper by R. Freter (11). A relevant portion appears as Appendix O.

Transfer of Foreign DNA from E. coli K-12. While the conversion of K-12 into a pathogen by the insertion of foreign DNA fragments appears impossible, the foreign DNA fragment could be transmitted to another bacterium with which the K-12 comes in contact, including other strains of E. coli. Such a transmission might result in insertion of the foreign DNA fragment into a known pathogen, or might convert the recipient into a pathogen, even if the original K-12 host cell does not multiply and live. Transfer of plasmids or bacteriophage, the vectors for the foreign DNA in K-12 systems, is a complex biological phenomenon. (See references 2 and 24 at the end of this section, and also the detailed discussion in Section III-A-1 of the Guidelines.) The following analysis of existing information indicates that with those K-12-vector systems permitted by the Guidelines, the likelihood of transmission is extremely remote.

The case of plasmid vectors is considered first. Certain plasmids are inherently able to transfer themselves from one bacterial cell to another. The Guidelines do not permit the use of such plasmids (called "conjugative" plasmids) for recombinant DNA experiments. Other plasmids only transfer themselves from one bacterial cell to another with great difficulty or not at all, and these plasmids (non-conjugative) may be used in recombinant DNA experiments.

However, a nonconjugative plasmid can be transferred much more readily if the cell harboring it is invaded by a conjugative plasmid. The conjugative plasmid confers upon the nonconjugative one the property of transferability. Therefore, even with a nonconjugative plasmid, the possiblity of transfer of the foreign DNA segment exists, though to a much lower extent.

The extent can be estimated by considering the probability that each of the necessary events takes place. There are extensive experimental data permitting such estimates: the probabilities differ depending on the particular plasmids being considered. With certain K-12 plasmid systems, it has been estimated that the maximum probability for transmission of a nonconjugative plasmid vector from a K-12 host cell is 1 in 10^{16} (that is, 1 in 10,000,000,000,000,000) K-12 surviving per day in the intestinal tract of warm-blooded animals (7, 8). The probability is even lower in sewers, sewage treatment plants, streams, and rivers. Most of the estimates of the probability of transfer of a nonconjugative plasmid are derived from data obtained under laboratory conditions. Knowledgeable experts believe that the probability will be even lower in natural environments, such as animal intestines (8). Nevertheless, the uncertainty indicates that animal and human feeding studies are required to verify the predictions (12). The extensive information on which these conclusions were based are summarized in reference 7.

The transfer of a foreign DNA fragment from the initial K-12 host to other bacteria by means of bacteriophage vectors also requires consideration.

Bacteriophage can escape from the laboratory either as mature infectious phage particles or in bacterial host cells in which the phage DNA is carried either as a plasmid or inserted within the cell's DNA (a well known example of natural recombination). The fate of K-12 host cells carrying the bacteriophage DNA as a plasmid or within the cell's DNA is similar to that for plasmid-containing host cells as discussed above. The survival of bacteriophage DNAs when released as infectious particles depends on their stability in nature, their infectivity and on the probability of subsequent encounters with naturally occurring E. coli strains sensitive to the bacteriophage. The probability of survival of those bacteriophage used in recombinant DNA experiments (called lambda) and their infection of resident intestinal E. coli in animals and humans is estimated to be small, given the high sensitivity of lambda to the acidity of the stomach, the insusceptibility of smooth type E. coli cells (the type that normally resides in the gut) to lambda infection, the infrequency of naturally occurring E. coli sensitive to lambda and the failure to detect infective lambda particles in human feces after ingestion of up to 10^{11} particles (15). Moreover, the particles are very sensitive to drying, as would occur if they escaped into the air.

Establishment of lambda as a stable insertion into the host cell genome is, in certain cases, a frequent event (10^{0} to 10^{-1} --i.e., 1 out of 1 to 1 out of 10) so that this mode of escape would be the preponderant laboratory hazard. However, most variants of lambda in use in recombinant DNA experiments (16, 17, 18), and all those variants to be certified as EK2 and EK3, have greatly reduced ability to become so inserted (probability of about 1 in 10^{5} to 10^{6} --100,000 to 1,000,000) (19, 20, 21). The frequency for the conversion of lambda to a plasmid state is also only about 1 in a million (22). Moreover, the routine treatment of bacteriophage samples with chloroform (23) should eliminate all surviving bacteria.

While not exact, the estimates for containment afforded by using bacteriophage host-vectors are sufficient to indicate that with currently employed systems the probability of transfer of a foreign DNA fragment from the original K-12 host to another bacteria is remote.

A more detailed discussion of the relevant properties of the bacteriophage lambda is found in Section III-B-1 of the NIH Guidelines. Additional information is in the following excerpt from reference 7.

"In order to obtain additional information on the likelihood of transmission of recombinant DNA, we have also commenced to collect a diversity of E. coli strains obtained from patients with bacteriemias, wound infections and urinary tract infections, from healthy individuals and from sewage. We have been examining these strains for the presence of nonsense suppressor mutations that would allow for the replication and perpetuation of lambda or plasmid vectors that contain nonsense mutations and also for the ability of these strains to be infected by lambda DNA that is tagged by an antibiotic resistance marker. So far, in a test of some 100 strains, we have failed to detect any strain with a nonsense suppressor or that was infectable by lambda. R. Davis, P. Leder and their colleaques have also examined some 2000 E. coli strains for sensitivity to phage lambda and although they found a few strains that appeared partially sensitive to lambda, none would propagate the virus."

One of the theoretical mechanisms by which organisms containing recombinant DNA might result in untoward effects is that the foreign DNA might be excised from the experimental or secondary host-vectors and recombined with cellular DNA of an animal harboring that host-vector [see Section IV-C-1-b-(2)]. The last paragraph of Appendix O deals with the probability of such events (11).

Thus far, the suitability of K-12 for recombinant DNA experiments has been considered in relation to the ability of the K-12 cells to cause any harm either directly or by transfer of a foreign DNA fragment to another bacterial cell. These properties will always depend on the ability of the K-12 to survive, multiply, and infect another living organism. The relative inability of K-12 to survive in natural environments has already been described. If it should survive and multiply, it is still unlikely to infect living things. E. coli bacteria are primarily spread by ingestion of food and water containing them; such agents are seldom spread by aerosols. It is estimated that about 10^6 to 10^9 (1 million to 1 billion) cells of pathogenic E. coli are required to cause disease (7, 13). Therefore, were a K-12 to become pathogenic, or to transfer a foreign DNA fragment to another bacteria that was or became pathogenic, then at least a million bacteria would be required to cause disease in a single individual.

The Guidelines emphasize protection of laboratory workers, as previously stated, because the individuals most at risk are those conducting experiments and because laboratory workers are the most likely carriers of recombinant DNA out of the laboratory. Should a laboratory worker carry out such agents, it would still be highly unlikely that any others would be infected, and the risk of a resulting epidemic is virtually nonexistent. There is abundant evidence for this assertion. We know from many years' experience that the separation of sewage from food and water supplies prevents epidemics of enteric bacteria such as E. coli.

The summary of the Falmouth Workshop on Risk Assessment (see Appendix M) states: "The mode of spread of enteric bacteria (viz., by the fecal-oral route) virtually eliminates the epidemic potential of any strain in areas where the water supplies are protected from contamination with raw sewage" (8). Further documentation of these points is found in the quotation on the following page by microbiologist Roy Curtiss (7).

There remains the question whether the insertion of a foreign DNA fragment into K-12 will significantly alter the properties of the latter with regard to survival and multiplication, or the ability of the plasmid and bacteriophage vectors to be spread. The improbability of thereby converting K-12 to a pathogen has already been discussed. Changes in ability to survive and multiply would be expected to involve not only the changes in the K-12 itself, or the plasmid or bacteriophage, but the nature of the environment in which it finds itself.

These questions are discussed in Section IV-C-2 of this EIS from a general point of view. Recently, additional data pertaining to the K-12 systems have been obtained. Those data relevant to lambda, the bacteriophage vector, indicate that insertion of foreign DNA fragments does not result in any increase in the efficiency of bacteriophage reproduction (14). Additional, more detailed information is given in reference 7.

Defective K-12 Systems. Theoretically, the most desirable bacterial recipient of recombinant DNA would be a species uniquely adapted to carefully controlled laboratory environments and unable to survive or transmit DNA to other organisms in any natural environment. This means that the bacterium should be unable to survive in normal ecological niches. It should be unable to establish itself as a long-lived and multiplying resident in or on living things, or in soil or water. In addition, these properties should not be significantly altered by insertion into the bacterium of recombined DNA. The organism should also, of course, lend itself to manipulation for successful execution of the proposed experiments.

No bacterium meeting all these requirements is known. Furthermore, it is possible that no such bacterium exists in nature. Available bacterial systems must be evaluated for relative safety and utility, depending on the extent to which they approach the ideal criteria. The foregoing summary of knowledge concerning K-12 and its known plasmids and bacteriophages indicates that these systems measure up very well when compared with the ideal criteria, and can therefore be recommended for use in certain recombinant DNA experiments. The Guidelines permit the use of such systems--EK1--for those experiments whose potential for hazard is considered to be nil, low, or minimal.

Excerpt from a letter by Roy Curtiss III to Donald S. Fredrickson, April 12, 1977.

In terms of communicability of E. coli K-12, we know that enteric diseases caused by enteropathogenic E. coli and various strains of Shigella, Salmonella and Vibrio are transmitted by contaminated food and water and that manifestation of disease symptoms requires consumption of approximately one million bacteria. Such enteric diseases are seldom spread by aerosols. Indeed, it is well known, for example, that cages of mice infected with Salmonella can be housed in the same room with uninfected mice which remain uninfected. The finding that E. coli cells can be recovered from the nasopharynx of approximately five percent of those humans tested might suggest that aerosol spread could occur. Such E. coli cells, however, are only intermittently present in the nasopharynx and are usually found at concentrations too low to initiate an infection even if they were representative of a pathogenic strain. They most likely get into the nasopharynx due to poor personal hygiene. After learning of these observations quite some years ago, I monitored my nostrils and skin for the presence of those E. coli K-12 strains I was working with. I was successful in detecting these strains about ten percent of the time when the monitoring was done at the end of the work day, but never obtained positive results when the monitoring was done the next morning. I should hasten to add that my research with E. coli K-12 at that time involved mouth pipetting and other aerosol-generating procedures on an open lab bench: procedures and conditions which are not permitted by the NIH Guidelines. These results, preliminary as they are, nevertheless suggest that E. coli K-12 does not colonize the nasopharynx. Based on these observations, the fact that E. coli's normal ecological niche is the colon and the fact that transmission of enteric diseases is by ingestion of contaminated water and food, I doubt that E. coli K-12 could be converted to an air-borne "infectious" agent by introduction of recombinant DNA. In terms of the more usual means for spread of enteric pathogens, it is evident that enteric diseases are very well controlled in the United States by sanitary engineering, even though there have been reports of poor water quality in some parts of the country and higher-than-desired levels of pollution of rivers, streams, etc. There is, however, a concerted effort to improve biological waste water treatment and thus lessen pollution and improve water quality. Even if there were a natural catastrophe such as caused by an earthquake, tornado, hurricane, etc., it is unlikely that E. coli K-12 containing recombinant DNA could initiate or sustain an epidemic in view of K-12's inability to colonize and overcome host defense mechanisms.

For many experiments judged to have a somewhat higher, though still only conjectural, potential for hazard, the Guidelines require even further attenuation of the K-12 system. Modifications of the properties of K-12 and the vectors are required, such that the survival of a genetic marker on the vector is less than 1 in 10^8 (100,000,000) except under specially designed and carefully regulated laboratory conditions (Guidelines, Section III-9). These are the so-called EK2 systems.

The criteria for certification as an EK2 system have been defined and enlarged during the past year, the first full year during which the Guidelines have been applicable. Extensive data are required and very demanding standards have been set in order that a system be certified by the NIH for use as an EK2 system. Design and construction of these organisms has been and is being carried out by NIH contractors and other interested investigators. Their use in recombinant DNA experiments is not allowed until they have been certified by the Director, NIH, upon recommendation by the Recombinant Advisory Committee. Appendix H to this EIS describes the criteria for certification and lists the certified EK2 systems. It should be noted that the same depth of experience with K-12 that recommends its utility as a host for recombinant DNA experiments is central to the ability to manipulate K-12 for the purpose of improving its safety.

c. Use of Simian Virus 40 (SV40) as carrier of a foreign DNA fragment

It has been argued that the use of SV40 as a vector should not be permitted, since this simian virus is known to cause cancer in laboratory animals. There is little evidence that SV40 can result in disease in humans. However, SV40 does infect humans, as indicated by antibody formation, and demonstrable antibodies to SV40 indicate that infection has occurred to some extent in the general population. Some of the infection may have resulted from the inadvertent inoculation of millions of persons during the initial mass immunization against polio virus, before SV40 was identified as a contaminant in the vaccine. Or the antibodies may have been formed against SV40-like viruses known to exist naturally in humans (25).

While it is conceivable that a recombinant DNA molecule containing the entire SV40 DNA plus a foreign DNA segment could infect humans and affect their health, this is extremely unlikely (26, 27, 28). The extensive knowledge of the genetics of SV40 virus shows clearly that it will not be possible to insert more than a very small piece of foreign DNA (less than a gene in size) into SV40 DNA without destroying its ability to form virus particles. The inserted piece will either replace essential genetic regions, destroy an essential gene by being inserted in the middle of it, or make the DNA molecule too big to be packaged into a virus particle. In addition to these intrinsic safety features, the Guidelines require that any recombinant DNA containing SV40 sequences be so constructed that it cannot reproduce independently as a virus. Much of the work will be done with only short pieces of the SV40 DNA which do not have the capacity to make any virus at all. Otherwise stated, this type of work will not be done with SV40, but only with portions of DNA derived from it. In addition, stringent physical containment is required.

d. Experiments involving the transfer of uncharacterized mixtures of DNA segments derived from warmblooded animals into bacteria

Experiments of the type indicated in this title are believed to present a greater possible risk than others because they involve a conglomeration of undefined genes that might include DNA capable of causing disease. For this reason the Guidelines require more stringent containment when such mixtures of DNA segments are used.

e. Use of oncogenic viruses

It has been argued that the introduction into E. coli of the whole DNA or any purified segment of the DNA of any virus that is oncogenic (i.e., cancer-causing) in any species should not be permitted. The Guidelines do prohibit the use of DNA from viruses that could cause cancer in man, as well as certain other viruses causing cancer in animals (Section III-A-i). Those viral DNAs that may be used are subject to the most rigorous containment requirements (Section III-B-2-c-i).

D. No Guidelines but NIH Consideration of Each Proposed Project on an Individual Basis Before Funding

With this alternative, individual investigators requesting NIH funds for projects involving recombinant DNA research would bring plans for proposed experiments to an NIH committee that would, without the use of formal guidelines, recommend suitable containment measures. Depending on the criteria used by the committee, this might result in lower or higher containment levels than the Guidelines currently impose. The advantages of such a procedure would include constant reevaluation of potential hazards and containment measures, and up-to-date information for investigators. The disadvantages include the enormous time and resources required for review, given the size of the biological research enterprise in the United States, the problem of finding knowledgeable individuals to serve on such a committee (essentially a full-time occupation), the opportunity for arbitrary decisions, and the bypassing of local input in assessment of hazards.

It should be pointed out that under the present NIH Guidelines, local institutional biohazards committees must consider proposed research projects on an individual basis and may impose more stringent safeguards than the Guidelines require. The judgments of the investigator and his local committee will be reevaluated by the NIH Study Section reviewing the scientific merit of the proposal.

E. General Federal Regulation of All Such Research

The NIH Guidelines are only binding for recombinant DNA research supported by NIH. Nevertheless, NIH has assumed responsibility to work toward the promulgation of safety measures for all such research. NIH conducted a series of meetings with representatives of other Federal agencies and private industry to insure as wide an application of the NIH Guidelines as possible. As a research agency, however, NIH does not have regulatory responsibility, and therefore, general Federal regulation was beyond its purview. Thus, the Secretary of HEW, with the approval of the President, established in October 1976 an Interagency Committee on Recombinant DNA Research, chaired by the Director of NIH. The Committee was chartered to review the nature and scope of Federal and private-sector activities related to recombinant DNA research, to determine the applicability of the NIH Guidelines to govern research in these sectors, and if necessary, to recommend appropriate legislative or executive action. The Committee consists of all Federal Departments and agencies that support and conduct such research and all regulatory agencies that may have potential authority over it.

Activities pertinent to the research agencies were reviewed by the full Committee in its initial meetings in the fall of 1976. All research agencies adopted the NIH Guidelines as standards, including Agriculture, Defense, the Energy Resources Development Administration, the National Aeronautics and Space Administration, and the National Science Foundation. Of these, only Agriculture and NSF (as well as NIH) are currently supporting and conducting recombinant DNA research. Also, at the November 23 meeting, the Federal regulatory agencies reported on their regulatory functions. Following that review, a special Subcommittee was formed to analyze the relevant statutory authorities for the possible regulation of research involving recombinant DNA technology. All regulatory agencies were represented on the Subcommittee, assisted by attorneys from their offices of general counsel. These included the Office of Safety and Health Administration, Environmental Protection Agency, Food and Drug Administration, Center for Disease Control, and Department of Transportation, and Department of Agriculture.

It was the conclusion of the Subcommittee that present law could permit some requirements to be imposed on much laboratory research involving recombinant DNA techniques, but that no single legal authority or combination of authorities currently existed that would clearly reach all research and other uses of recombinant DNA techniques and meet all stated requirements of the Committee. Although certain existing authority might be interpreted broadly to cover most of the research at issue, it was generally agreed that regulatory actions taken on the basis of any such interpretation would probably be subject to legal challenge.

The full Committee adopted the report of its Subcommittee and agreed that new legislation was required.

In considering the elements for legislation, the Committee reviewed Federal, State, and local activities bearing on the regulation of recombinant DNA research. There was a concerted attempt to obtain information of relevant activities in developing Federal policy, including review of the recommendations by the New York State Attorney General's Environmental Health Bureau for State regulation and the Cambridge (Massachusetts) City Counsel's resolution on recombinant DNA research.

Among Congressional proposals reviewed were S. 621, "The DNA Research Act of 1977," introduced by Senator Dale Bumpers, and the companion measure introduced by Representative Richard L. Ottinger in the House (H.R. 3591). The Committee also noted the resolution introduced by Representative Ottinger on January 19, 1977 (H. Res. 131), requesting DHEW to regulate recombinant DNA research under Section 361 of the Public Health Service Act.

Several committee representatives also reported on meetings with other interested parties whose views had been solicited on legislation to regulate recombinant DNA research. Those who were contacted include agricultural scientists, biomedical scientists, environmentalists, labor unions, and private industry. At Dr. Fredrickson's request, the Industrial Research Institute and the Pharmaceutical Manufacturers Association conducted a survey of their member firms to determine the scope of the research efforts in the private sector. The Pharmaceutical Manufacturers Association has endorsed the NIH Guidelines as standards for conduct of this research.

In considering elements of proposed legislation, a number of issues were raised and discussed fully by the Committee. After detailed deliberations at meetings on March 10 and 14, 1977, the Committee agreed on a set of elements for proposed legislation. The elements agreed upon and the various alternatives reviewed by the Committee were presented in an Interim Report transmitted to HEW Secretary Califano on March 15, 1977. The report of the Interagency Committee is included in Appendix I.

HEW Secretary Califano had legislation developed in light of the Committee's recommendations. The Administration bill drafted by the Department was reviewed by all members of the Interagency Committee and, at the request of the Office of Management and Budget, by all Departments and agencies. The bill was introduced by Senator Edward M. Kennedy, Chairman of the Subcommittee on Health and Scientific Research of the Senate's Committee on Human Resources, and by Representative Paul G. Rogers, Chairman of the Subcommittee on Health and the Environment of the Interstate and Foreign Commerce Committee. Congressional hearings have been held, and both the House and Senate bills are pending. A copy of the Administration bill as originally introduced is included as Appendix N.

Further, the Federal Interagency Committee on Recombinant DNA Research is currently addressing international aspects of recombinant DNA activities. The Committee is reviewing the nature and scope of international scientific activities related to recombinant DNA research, with particular attention to the setting of safety standards for recombinant DNA activities by national and international health and safety organizations. It is expected that the full committee will conclude its review and prepare a report with recommendations, if any, to the Secretary, HEW, in the early fall.

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VII.
ENVIRONMENTAL IMPACT OF THE GUIDELINES

A. Impact of Issuance of NIH Guidelines

The primary impact of issuance of the Guidelines is to provide a mechanism for the protection of the laboratory worker, the general public, and the environment from the possible hazards that might result from recombinant DNA molecule research. Such research is defined as that which involves molecules consisting of segments of deoxyribonucleic acid (the material that determines the hereditary characteristics of all known cells) which have been joined together in cell-free systems and which have the capacity to infect and replicate in some host cell. The Guidelines establish carefully controlled conditions for the conduct of experiments involving recombinant DNA.

Any unusual hazards of this research remain hypothetical at present; the speculations on risks that underlie the Guidelines may prove to be wrong, and it may be that no potential for hazard exists. Nevertheless, the Guidelines take cognizance of the possibility of dangers to the laboratory worker, other persons, animals, plants, and the environment posed by the emerging research technology involving recombinant DNA molecules. They call for a number of measures aimed at reducing or eliminating human and environmental exposure to materials containing recombinant DNA molecules, in case they should prove hazardous.

The NIH recognizes that the production of unexpected results is inherent in the very nature of scientific research. The danger of exposure to certain of the conceivable hazards as a result of NIH-sponsored research on recombinant DNA is eliminated by the prohibition of certain experiments. The danger of exposure to other conceivable but hypotheical hazards is reduced by the containment requirements. A totally risk-free situation, however, was not the aim of the Guidelines, nor will it be provided by adherence to them. In the view of NIH, the level of risk that will result from adherence to the Guidelines is extremely small. But views to the contrary have been expressed. There have been arguments to the effect that the level of acceptable risk assumed in the Guidelines is too high, and arguments that it is too low. As a matter of policy, the Guidelines will be subject to constant reassessment in the light of new knowledge and the changing views of scientists and the public. A variety of channels are available for expression of views and action, including the institutional biohazards committees, the NIH Recombinant Advisory Committee, and the NIH Director's Advisory Committee. There has been extensive opportunity for public debate and participation at the State and local governmental levels.

With regard to the anticipated but speculative benefits of recombinant DNA research, adherence to the Guidelines may postpone their realization. Certain experiments are prohibited. The Guidelines are binding on all NIH grantees and contractors as well as NIH intramural scientists and laboratories. The Guidelines serve as a model for all other Federally supported work and comparable activities in the private sector. All work with recombinant DNA techniques known by NIH to be occurring in this country is presently being carried out under these guidelines.

1. Impact on the Safety of Laboratory Personnel and on the Spread of Possibly Hazardous Agents by Infected Laboratory Personnel

The NIH Guidelines are directly concerned with reducing and eliminating exposures of laboratory personnel and all other persons to host cells and microorganisms containing recombinant DNA molecules. The degree to which the potential for exposures are reduced or eliminated corresponds to the assessed potential hazard of the experiments. The NIH Guidelines classify allowable experiments into four levels of potential hazard. And for each level of experiments, the Guidelines describe safe laboratory practices, containment equipment, and facilities (Section II).

The four levels of physical containment are based on recommended methods for the safe handling of microorganisms that produce human disease of varying degrees of severity. The descriptions of these requirements were developed by a working group of biological safety experts representing the Center for Disease Control (CDC), the National Institutes of Health, the National Animal Disease Laboratories (Department of Agriculture), and the Frederick Cancer Research Center (FCRC) of the National Cancer Institute, NIH. Representatives from FCRC were previously responsible for directing the biological safety programs at Fort Detrick. The names of these experts are listed at the end of Appendix D of the NIH Guidelines.

The protection of laboratory personnel is achieved through the use of host-vector systems that are not infectious to man as well as the use of safe laboratory practices and containment equipment. The capability of safe laboratory practices and containment equipment for protecting laboratory workers can be assessed by evaluating their effectiveness when used for the containment of human pathogens. The documented experience at Ft. Detrick for the 25-year period during which human pathogens capable of causing serious disease in man were extensively handled (while safe laboratory practices and equipment were improved) provides a basis for such an evaluation.

The frequency of laboratory-acquired infections for four periods of operation at Ft. Detrick are summarized in the table below. These periods were selected because the safe laboratory practices and containment equipment used during these periods approximate those required by the NIH Guidelines for P1, P2, P3, and P4 physical containment levels. In considering this experience, it must be emphasized that the majority of agents handled at Ft. Detrick are now classified by the CDC as class 3 and class 4 human pathogens, the use of which is prohibited by the Guidelines in recombinant DNA research.

ESTIMATED LABORATORY-ACQUIRED INFECTION RATES AMONG FORT DETRICK LABORATORY PERSONNEL

Period	Group at risk	Approximate physical containment level	Laboratory-acquired infections per million man-hours worked*
1943-45	All laboratory-admitted personnel**	P1	35[1]
1954-58	All laboratory-admitted civilians	P2	9[1]
1960-62	All laboratory-admitted civilians	P3	2[1]
1960-69	All laboratory-admitted personnel in Building 1412B	P4	1[2]

*Includes subclinical infections and mild illnesses where hospitalization was not required. **During this time, personnel were predominantly military rather than civilian; after 1946 the reverse was true.

This experience demonstrates a reduction in laboratory-acquired infections with increasing levels of physical containment. It also demonstrates that when known hazardous agents are handled, the risk of a laboratory-acquired infection cannot be totally eliminated.

The risk of laboratory-acquired infections can be placed in clearer perspective when compared with the risk of loss-time or disabling injuries experienced in other occupations. For example, the rate of disabling injuries of all kinds at Ft. Detrick in 1964, including hospitalized laboratory-acquired illnesses, was 0.49 per million man-hours worked. This compares favorably with the experience for the same period in other industries as reported by the National Safety Council. The disabling injuries per million man-hours worked in 1974 for several industries were as follows: communication industry, 1.20 (lowest of all reported); automobile industry, 1.86; chemical industry, 3.12; Federal Government, 7.3; printing and publishing industry, 9.35; lumber industry, 17.76; air transport industry, 18.77; and mining industry, 37.78. This experience suggests that research involving human pathogens, where the hazard is recognized and safe practices and containment equipment are used, is a relatively safe occupation.

It is important to emphasize the role of containment equipment in protecting the laboratory worker from potential exposures to microorganisms containing recombinant DNA molecules. Analysis of comprehensive surveys of laboratory-acquired infections (3, 4, 5) demonstrates that fewer than 20 percent of known infections can be attributed to a documented accidental exposure. The knowledge that most microbiological practices create aerosols (6) suggests that inhalation of undetected aerosols may contribute significantly to occupational illness among laboratory workers who handle human pathogens. It is on this conclusion that physical containment equipment is required by the NIH Guidelines for control of potential aerosol exposures. Thus, as the assessed hazard level increases from P1 to P4, the NIH Guidelines require greater use of physical containment equipment. For example, procedures that have the potential for producing considerable aerosols are required to be isolated by physical containment equipment when these procedures involve microorganisms that require P2 containment. All procedures involving moderate-risk materials (P3), however, are required to be physically contained or isolated in such equipment, and all procedures involving high-risk materials (P4) are required to be contained in gas-tight biological safety cabinets.

Open-front biological safety cabinets are used to contain organisms requiring P2 and P3 levels of physical containment. Personnel protection against aerosol exposures is provided by the movement of air into the cabinet through the open front. This air flow has an average velocity of at least 75 feet per minute. These cabinets are designed so that fewer than 20 microorganisms will escape through the open front when 1×10^8 (100,000,000) to 8×10^8 (800,000,000) microorganisms are experimentally released within the cabinet.

The capability for preventing exposures to experimental materials is greatest with the use of class III biological safety cabinets. These cabinets are required for all experiments requiring P4-level physical containment. Class III cabinets are gas-tight, stainless steel, ventilated enclosures, usually of a modular design, with attached arm-length rubber gloves. The cabinets are ventilated at a rate of 10-20 air changes per hour, with the supply and exhaust air passing through HEPA (high-efficiency particulate air) filters. Provision for exhaust air incineration is usually provided, and all liquid wastes from the cabinets pass through a secondary sewage sterilization system. The cabinets are usually equipped with compressed air, vacuum, hot and cold water, ultraviolet lights, and any desired array of back- or bottom-mounted refrigerators, incubators, deep freezers, centrifuge cabinets, necropsy cabinets, and animal-holding cabinets.

"Gas-tight" means that the cabinets were pressurized to three inches water-guage pressure with dichlorodifluoromethane, and all joints, junctures, attachments, and the rubber gloves were tested with a halogen detector. The NIH Guidelines require that under these test conditions there shall be no leak rate greater than .01 oz. of gas per year. When used to contain microorganisms, the class III cabinets are operated under a reduced pressure of from 0.5 to 1.0 in. of water guage. This operating condition provides an additional safety factor.

Gloves are either 15 or 30 mil. thick. They are produced by a multiple-dip cement process and electrically tested at 5,000 or 10,000 volts, depending upon thickness, to detect any defect.

Class III cabinets provide, under ordinary circumstances, an impenetrable barrier between the inside of the cabinet and the laboratory environment. However, it is recognized that human error and accidents can compromise the integrity of class III cabinets. Attached rubber gloves have been torn, improper techniques in changing gloves have been reported, contaminated materials have been improperly removed from the cabinets, and fires and explosions have occurred in these cabinet systems. Scientists and technicians have injected themselves through the gloves when using a syringe and needle, and animal caretakers have received animal bites through the gloves. Although these situations have been observed, they occur infrequently. This is exemplified by the fact that in Building 1412B at Ft. Detrick, where P4 conditions were used for 10 years, there was only one laboratory-acquired infection. This was caused by a needle puncture through an attached glove.

Biological safety cabinets and other containment equipment are not required for work at the P1 level. It is recognized that under these conditions, exposures to aerosols may occur. However, P1 conditions are only allowed for experiments involving organisms that are known to possess no or minimal hazard to man and that exchange genetic information in nature. Prokaryotic and eukaryotic host organisms that will be used in the conduct of these experiments have traditionally been handled on the open bench, yet they have never been associated with any laboratory-acquired infection. Indeed, out of the 3,921 laboratory-acquired infections reviewed by Pike (5), only 166 were caused by class I agents, of which 161 infections were superficial skin irritations caused by fungi.

The Ft. Detrick experience discussed above provides an extremely conservative basis for assessing the capability of the safeguards required by the NIH Guidelines for recombinant DNA research. For example, it would be expected that the rate of laboratory-acquired infections indicated for P1 conditions would have been considerably lower if these conditions were only used for class 1 and class 2 organisms rather than for class 3 and class 4 organisms.

For example, enteric pathogens classified by the CDC as class 2 agents are only a primary a hazard to the laboratory worker if large quantities of the organisms are ingested. The infectious dose--that is, the number of organisms ingested--necessary to cause typhoid fever, cholera, and shigellosis in 25 to 50 percent of volunteers has been reported to be 10^5 (100,000) (7), 10^8 (100,000,000) (8), and 10^9 (1,000,000,000) (9) respectively. These diseases are not known to be caused by exposures to infectious aerosols. On the other hand, most class 3 and class 4 agents can cause infection through inhalation of infectious aerosols. Of even greater importance, agents responsible for most documented laboratory-acquired infections have the potential to cause disease when fewer than 50 organisms are inhaled [Q fever (10), tularemia (11)]. Also, equivalent P4 safeguards were only employed when research was conducted that required the experimental generation of highly concentrated aerosols. Thus, the Ft. Detrick experience should be interpreted as a measure of the potential for aerosol exposure to research materials rather than as an indication of potential risks of conducting recombinant DNA research. Nevertheless, this experience demonstrates that P3- and P4-level safeguards can protect the laboratory worker from infection where research is being conducted with agents known to cause serious disease in man.

The laboratory worker is also protected by the restrictions placed on the sources of DNA and the host and vectors that can be used in recombinant DNA experiments. The Guidelines prohibit the use of organisms as DNA donors if they possess intrinsic hazards greater than that possessed by a class 2 organism. The host organism in which the recombinant DNA molecules will be inserted must at least conform to the definition of a class 1 etiologic agent. Thus, the host organism containing recombinant DNA molecules must acquire the virulence, transmissibility, and pathogenicity that neither host nor donor organism inherently possess if it is to create a risk similar to that of a class 3 or class 4 pathogen. Such acquisition would be an event unparalleled in the history of microbiology.

If host organisms containing recombinant DNA molecules were to acquire an unusual hazard potential, it is likely that such hazard would only be expressed following the ingestion of large quantities of the organism. This mechanism of exposure can be eliminated by the use of safe pipetting practice and personal hygienic measures common to our society.

The safeguards required for the conduct of recombinant DNA research demonstrate considerable caution. They are more than adequate to protect the laboratory worker conducting experiments involving recombinant DNA molecules.

The experience in research with human pathogens also demonstrates that the potential for an accidentally infected laboratory worker to transmit disease to another person outside the laboratory is exceedingly small. It has only been possible to document eight infections that were presumably transmitted to other persons from laboratory-infected workers. A wife contracted Marburg disease 11 weeks after her husband's recovery from a laboratory-acquired infection (12). A wife was also reported to have acquired Bolivian hemorrhagic fever after visiting her husband in the hospital (13). Recently in England, fatal smallpox was transmitted to two visitors by a hospitalized vaccinated laboratory technician mildly ill with smallpox undiagnosed at the time of the visit (14). In four European surveys of laboratory-acquired infections in which 265 infections of typhoid, paratyphoid, shigellosis, and salmonellosis were reported, the authors observed four secondary cases. In two cases typhoid fever was contracted by a patient's nurse (15); in one case typhoid was contracted by a patient's residential roommate and in one case the wife of a patient contracted cholera. An important aspect of each survey is that the survey questionnaire included a query as to whether there were secondary cases. In addition, the examination of laboratory-acquired infections data from the Center for Disease Control (CDC), National Animal Disease Center (NADC), NIH and Ft. Detrick reveals no case where disease was transmitted from an infected laboratory worker to another person.

Laboratory workers, of course, may become infected with pathogens that are not under study. For example, viral hepatitis in laboratory workers has been reported on numerous occasions (5). Infections

occurred because the workers, mainly in diagnostic laboratories, were unaware of the virus in their samples.

2. Impact on the Environmental Spread of Possibly Hazardous Agents

The NIH Guidelines are directly concerned with preventing the release of cells and microorganisms containing recombinant DNA molecules, or the release of such molecules themselves, into the environment, thus preventing potential exposures of humans, other animals, and plant communities.

The Guidelines require decontamination of all liquid and solid wastes generated by low-, moderate-, or high-risk experiments. As the assessed risk of these materials increases (low → high), further measures are required to increase the certainty of containment. The Guidelines recommend the decontamination of no- or minimal-risk materials before their disposal to the environment. This is a standard microbiological practice.

The Guidelines prohibit the release of contaminated air under ordinary conditions. Procedures involving low- and moderate-risk materials that may produce aerosols are confined to primary barriers. Contaminants in the exhaust air from these barriers are removed by filtration.

The potential for accidental release of recombinant DNA materials into the atmosphere, however, increases with decreasing containment requirements (moderate → minimal). Harmful secondary effects from such accidental release of minimal-, low-, or moderate-risk materials are exceedingly remote. An analysis of 36 reported laboratory-acquired micro-epidemics in the period 1925-1975 involving over 1,000 infections with human etiologic agents of classes 2, 3, and 4 demonstrated no infections among persons who were never in the laboratory building or who were not associated in some way with the laboratory (2). Almost all of these outbreaks occurred in the absence of genuine efforts to control contaminated air, liquid wastes, refuse, and laundry.

Adherence to the NIH Guidelines should prevent any potential release of high-risk materials to the environment. All high-risk materials are required to be isolated in physically contained, absolute primary barriers. All effluents from these barriers are sterilized. The barriers themselves are located in maximum-security facilities, which are provided with additional barriers to prevent any accidental release. Air locks, negative air pressure, clothes-change rooms, filtration and incineration of all air exhausted from the facility, and the secondary sterilization of all liquid and solid wastes provide additional protection to the environment.

The NIH Guidelines also define requirements for protecting the environment from potential dangers that may be associated with the shipment of recombinant DNA materials. Federal packaging standards appropriate for the shipment of class 4 human etiologic agents are required for the shipment of all recombinant materials.

3. Cost Impact

The direct cost impact of the NIH Guidelines is the cost of complying with their provisions. The costs will vary according to the level of potential risk of the research. There are no special facility requirements for work with minimal- and low-risk recombinant DNA materials (P1 and P2 containment). There are equipment requirements for work involving low-risk recombinant DNA materials that will involve little cost impact. Low-risk research requires a biological safety cabinet for procedures that may produce significant aerosols and an autoclave for sterilizing waste materials. These items of equipment, however, are generally available within the existing facilities where such research is being conducted. The cost impact of the NIH guidelines on minimal- and low-risk research is therefore not significant.

Special equipment and facility requirements are specified for moderate-risk recombinant DNA research (P3). All work at this level of potential risk is to be conducted within class I or II biological safety cabinets. This requirement will necessitate the acquisition of many additional cabinets, depending on the scope of the research effort. It is estimated that one cabinet, costing approximately $5,000, will be required for every three persons involved in the research.

Directional air flow, single-pass ventilation, and provisions for ensuring restricted access are specified requirements for moderate risk (P3) recombinant DNA research. While many new facilities (those constructed in the last decade) have this capability, few older facilities can provide it without extensive renovation. Creating adequate access control by construction of architectural barriers (air locks, double-door alcove, etc.) is not expensive. However, the cost of renovating air-handling systems to provide for single-pass, directional air flow may prevent some institutions from conducting moderate-risk research. It has been estimated that installation of air-handling systems that comply with the NIH Guidelines would cost approximately $200 per square foot of space serviced by the system.

The NIH Guidelines require that high-risk (P4) research involving recombinant DNA materials be conducted only in class III biological safety cabinets (glove boxes) that are installed in maximum-security facilities. Fewer than 30 facilities within the United States have the potential for meeting the requirements. A smaller number may actually be available for this research. The cost of constructing and equipping a maximum-security facility having two 10-foot by 20-foot laboratory modules with class III cabinetry is estimated at $750,000. This high cost is due to sophisticated mechanical support systems (e.g., negative pressure, exhaust air filtration, air-waste treatment plant), and architectural barriers (e.g., clothes-change rooms, air locks, waste-staging areas, and monolithic walls, floors, and ceilings). The cost of class III cabinetry installed is approximately $3,000 per linear foot. In addition, the cabinetry line and the facility each require a double-door autoclave, costing a minimum of $15,000 and $65,000 respectively.

4. Secondary Impacts

Issuance of the NIH Guidelines will have three secondary impacts in terms of environmental protection--i.e., will reduce the potential risk to the environment from recombinant DNA research:

a. Limited maximum-security containment capability

Only a small number of maximum-security facilities are capable of meeting, or being modified to meet, the requirements of the Guidelines. The Guidelines therefore reduce the number of "High-risk" recombinant DNA experiments that could be conducted were the Guidelines not imposed.

b. Safety awareness

The safe performance of biomedical research is dependent on an awareness of the risks and on the safeguards required to control the risks. Issuance of the NIH Guidelines should strengthen safety performance in general by providing safety information and increasing the laboratory worker's awareness of potential hazards and the correct procedures for dealing with them.

c. Early recognition of unforeseen hazards

The Guidelines require that the principal investigator notify NIH of any serious or extended illness or accident that may result in serious exposure to man or to the environment. This monitoring procedure will provide an early warning of possible unforeseen hazard. For example, if a laboratory infection from exposure to a recombinant DNA molecule is confirmed, indicating a real hazard, an increase in safeguards or cessation of experiments can be required to minimize the hazard to other investigators conducting similar studies. This provision will also reduce any potential for environmental effects.

B. Impact of Experiments Conducted Under the Guidelines

1. Possible Undesirable Impact

a. Dispersion of potentially hazardous agents

The hypothetical mechanisms by which insertion of foreign genes into cells or viruses might result in the formation of hazardous agents are described in Section IV-C of this EIS. The mechanisms are, in principle, applicable to persons, animals, and plants. There is, as stated before, no known instance in which a hazardous agent has been created by recombinant DNA technology. Current knowledge permits no more than speculation that such agents may be produced and an equally speculative assessment of the nature and extent of hazards associated with a particular recombinant DNA experiment. This is the underlying reason that the thrust of the Guidelines is to minimize contact of organisms containing recombinant DNA with other organisms or the environment. Therefore, the following analysis of possible undesirable impacts due to dispersion of potentially hazardous agents emphasizes the likelihood of significant dispersion rather than the nature of the hazard itself. The analysis given does not apply in detail to all possible situations, but can serve as a model for analyzing different ones.

In order that any potential hazard be realized, it is necessary that each of a number of sequential events occur. Each event in the sequence is possible only if the earlier events have occurred. The organism must--

(a) contain foreign genes,

(b) escape from the experimental situation,

(c) survive after escape,

(d) become established in an environment permitting its growth and multiplication,

(e) contact other living organisms in a significant manner, including contact by a sufficient number of organisms to ensure survival and growth and to cause infection. [Note that the environment in (d) may be a living organism itself.]

In those cases where the detrimental effect would result from the formation of a harmful protein, the organism containing the recombinant DNA would have to--

(f) contain a gene for a potentially harmful protein,

(g) be able to express the foreign gene (that is, synthesize the corresponding protein) and

(h) synthesize the protein in sufficient quantity to be deleterious to the infected organism.

The overall probability of a detrimental effect resulting from the formation of a harmful protein is then equal to the product obtained by multiplying together the probability of each event, (a) through (h).

In those cases where the foreign DNA itself might be the cause of undesirable effects, another set of events must be considered. Where the foreign DNA is presumed to increase the pathogenicity of the initial host cell or virus, the inserted DNA must--

(i) impart a selective advantage for growth to the carrier of the recombinant DNA as compared with the original cell or virus,

(j) alter the metabolism of the carrier so that it becomes disease-producing.

The overall probability of a detrimental effect by this mechanism is then equal to the product obtained by multiplying together the probabilities of events (a) through (e) times (i) through (j).

In the case where the foreign DNA is presumed to cause undesirable effects by virtue of its transfer out of the original recipient and reinsertion into cells of another species, the DNA must--

(k) leave the original recipient without being destroyed,

(l) survive transfer to another cell,

(m) become associated with the other cell in a stable manner, either as an independent element or by natural recombination.

The overall probability of an undesirable effect arising by means of the secondary transfer mechanism is equal to the product obtained by multiplying together the probabilities of events (a) through (e) and (k) through (m).

It is currently impossible to calculate accurately the overall probabilities for most experiments. But crude estimates can often be made for the specific events. The probability of event (b) can be estimated from the nature of the physical containment practices and procedures and from specific knowledge of accidental release due to failure of equipment or technique. An idea of the probability of events (c) and (d) may be obtained from the known properties of the host-vector system in the particular experiment. Some estimate of event (e) may be made from the known properties of the host cell. For events (f) the details of the particular experiment--for example, whether it is a shotgun experiment or not--will be helpful in obtaining a range of probabilities. As more data are accumulated, some idea of the probabilities of events (g) and (h) will be possible. Again the details of the particular experiment, and the relation between the foreign DNA and the new host, will be relevant. (See Section IV-C-1-a of this EIS for a discussion of expression of foreign gene in recipient host cells.) It may also be possible to make some estimate of the probability of event (i) in any given case. Estimates of the likelihood of event (j) will be among the most difficult, given the complex and poorly understood nature of what makes any agent a producer of disease. Useful though approximate estimates of events (k), (l), and (m) can be obtained from prior knowledge of the properties of the vector, including its transmissibility, stability, and ability to enter other cells and establish itself therein. (See Section VI-C-3-b of the EIS and Section III-B-1 of the Guidelines.)

It is not necessary to know the probability of each event to make useful estimates. If the probability of a few of the necessary events is known with some confidence, useful numbers can be obtained. In such a case, even if the probability of all the other events is assumed to be high--even equal to 1 (that is, a certainty)--an upper limit of the overall probability can be obtained. It must be recognized that the description of an overall probability as very low, or low, or high is relative and depends on how many organisms are present. Thus, the overall probability of a detrimental effect will vary directly with the number of organisms prepared.

The only article of which we are aware that has been published in the scientific literature attempting to assess numerically the probability of a man-made epidemic resulting from recombinant DNA research was written by Dr. Robin Holliday, FRS, Head of the Division of Genetics, National Institute of Medical Research, London (16). Excerpts from Dr. Holliday's article appear as Appendix P.

Virologists of the National Institute of Allergy and Infectious Diseases are preparing to conduct experiments at the Frederick Cancer Research Center, Frederick, Maryland, which should provide data to help determine if recombinant DNA research could result in real hazards.

b. Potential long-term evolutionary consequences

Section IV-A of this EIS describes the mechanisms by which DNA segments may be moved from one organism to another, either within a given species or between species. As pointed out there, natural recombinations across species lines have been observed between a limited number of microorganisms. Natural recombination between single-celled organisms such as those used as host cells in recombinant DNA experiments and more complex animals and plants has not been observed.

It has been suggested that the latter recombinations do not, or cannot, occur (17, 18) or else occur very seldom (19). Further, it has been argued that the formation of recombinants between distantly related species in the laboratory, and the accidental release of such recombinants into the biosphere, might lead to unpredictable evolutionary consequences (17, 19). Because the detailed processes of evolution are not well understood, the nature of those possible consequences has not been and cannot be described. Similarly, no mechanism by which a recombined DNA would alter evolution has been suggested.

On the other hand, it has been argued that evolutionary consequences of recombinant DNA research are not likely to be serious (20). This argument states that natural recombinations between distantly related species are probably more frequent than available data would suggest--that there is no 'barrier' between prokaryotes and eukaryotes. Natural recombinants have not been observed because no one has looked for them. Opportunities for such recombination are frequent in nature, since prokaryotes live in close proximity to and, in some instances, within cells of eukaryotes. And given the likelihood that such recombinants will usually be at a selective disadvantage (see Section IV-C-1-a of this EIS), they would be short-lived and hard to find. The argument concludes that man-made recombinants are probably not novel and are not likely to lead to novel evolutionary consequences.

The Guidelines recognize this issue by requiring stricter containment for experiments involving organisms not known to exchange DNA in nature than for those involving organisms known to do so (Section III-B-2-ii of the Guidelines). But the indefinite and remote nature of the concern indicates that more restrictive action is unwarranted.

c. Other considerations

The foregoing descriptions of the kinds of possibly hazardous situations that might arise from organisms obtained through recombinant DNA experiments must be considered in the light of certain more general issues.

(1) Monitoring for release of organisms containing recombined DNA

Control of the spread of any agent outside an experimental situation to laboratory workers or the outside environment is greatly assisted by adequate means for monitoring the agent in question. An example is the monitoring for spillage and spread of radioisotopes. The presence of radioisotopes is readily measured, and the exposure of laboratory personnel or the environment to radiation can be quantified. In the case of organisms containing recombined DNA, the situation is fundamentally different. No simple general procedure exists for identifying an organism released from the laboratory against the large background level of related and unrelated organisms occurring naturally.

It is possible, however, to devise special pertinent procedures for detection of some of the agents used in recombinant DNA experiments (21). For example, development of bacterial strains, phages, or plasmids carrying readily detectable genetic traits would enable the monitoring of laboratory personnel, people working in the area, and their families for the presence of those agents. This would be analogous to the examination of drinking water, lakes, etc., for fecal contamination with enteric organisms. Detection in such instances could be at levels as low as 10^{-7} (1 part in 10,000,000). The adequacy of such screening is not presently known.

Given a series of events that might characterize a hazardous situation, the time factors involved become relevant. Certain types of organisms containing recombinant DNA, if they escaped and were hazardous, might immediately be perceived as such--e.g., through their production of toxic foreign proteins. We might therefore be aware of the potential problem soon after dispersal of the organism, and reasonable means for minimizing further dispersal could be undertaken. In other hypothetical events--e.g., the escape of a cancer-producing DNA fragment--evidence of harmful effects might not be apparent for many years. Moreover, the connection between the causative organisms and the observed harmful effects could be difficult to establish.

(2) Natural occurrence of DNA recombination between unrelated organisms

Concern over the potential for hazard in organisms containing recombined DNA develops from the central idea that such recombinants will be unique types of organisms, not normally arising in nature, and that their properties will therefore be unknown and unpredictable. Natural environments provide many opportunities for recombination of DNA between unrelated species, as for example, in the intestines of animals [see Section IV-C-1-b-(2) of this EIS]. Whether, or at what frequency, such recombinations occur is not known at present. They are probably rare, however, given the very low extent of shared base sequences that can be detected in DNAs derived from distantly related organisms. It would appear that naturally occurring interspecies recombinants, if they occur in nature, may have been selected against in evolution. However, tests for shared base sequences are of limited sensitivity.

(3) Relative irreversibility of spread of organisms

Should organisms containing recombined DNA be dispersed into the environment, they might, depending on their fitness relative to naturally occuring organisms, find a suitable ecological niche for their own reproduction. A potentially dangerous organism might then multiply and spread. Subsequent cessation of experiments would not stop the diffusion of the hazardous agent.

As described earlier, however, it is likely that newly constructed organisms would be less fit than those occurring naturally and would therefore disappear over time.

d. Views of the possible undesirable impact

We quote in its entirety (following page) an article by Rene Dubos, Professor Emeritus at Rockefeller University, as it appeared in the New York Times on April 21, 1977 (22).

Next, we quote from the article "Recombinant DNA: Fact and Fiction" by Stanley N. Cohen, Science, February 18, 1977 (23).

Finally, we quote an editorial (p. 107) by Richard Goldstein from the New England Journal of Medicine and an article (Appendix Q) from the same issue, "When May Research Be Stopped?" by Carl Cohen, dated May 26, 1977 (24).

"Genetic Engineering" by Rene Dubos, quoted in entirety from The New York Times, April 21, 1977. Copyright © 1977 by the New York Times Company. Reprinted by permission.

Genetic engineering is not new. It began in 1930—almost half a century ago—when a technique was developed at The Rockefeller Institute Hospital to modify in the test tube the hereditary characteristics of the microbes that cause lobar pneumonia. This achievement led to the demonstration that the substance now known as DNA (deoxyribonucleic acid) is the carrier of heredity.

(Although I was present at the birth of genetic engineering, I never worked in the field and can only marvel at the new techniques of recombinant DNA that now make it possible to transfer genes from any kind of living thing to any other kind.)

In reality, it is misleading to state that "scientists are now able to create new forms of life." The genetic engineering of today means only the introduction into fairly simple microbes of a few genes derived either from other microbes or from higher forms of life, including man. However, the very fact that such genetic combination is possible has created hope and anguish—hope that the technique will be used to beneficial purposes, anguish at the thought that it might generate new diseases and lead to the manipulation of human nature.

For a long time, I had a quasi-religious hostility to experiments combining genes from different organisms, because I felt that this was contrary to the ways of nature. Like other biologists, I used to believe that gene exchange does not take place in nature, except during sexual conjugation between creatures of the same species. I now realize, however, that genetic exchange occurs frequently under natural conditions. For example, harmless bacteria readily incorporate gene fragments from other bacteria and thus become able to produce toxins, to resist antibiotics, to cause cancer in plants.

Contrariwise, virulent bacteria can lose the genes that make them dangerous. It is likely that bacteria also can incorporate DNA fragments from the animals and plants in which they reside.

Since gene exchange occurs widely in nature, I now feel that it is proper to do it experimentally under controlled conditions.

Even though human genes can be incorporated into bacteria and other organisms, changing human nature by genetic engineering seems to me impossible except perhaps for the correction of a few genetic maladies. Furthermore, my long experience in the field of infectious diseases has convinced me that laboratory techniques are most unlikely to produce strains of microbes that will start worldwide epidemics of new forms of disease. Billions upon billions of microbes exist everywhere in nature, constantly undergoing genetic changes, yet only very few can cause disease, and even then only when conditions are just right.

The great epidemics of the past and the increase in certain forms of cancer among us can be traced more to environmental factors than to new strains of microbes or viruses. For example, the severity of the 1918 flu pandemic was largely due to the conditions created by World War I.

I doubt that gene recombination in the laboratory will create microbes more virulent than those endlessly being created by natural processes. In any case, we know a great deal about handling dangerous microbes. I have been in daily contact on smallpox, rabies, typhus, plague, cholera, tuberculosis, etc., yet have witnessed very few laboratory infections and never one responsible for starting an epidemic.

Like all human enterprises, genetic engineering may entail some unpredictable risk. I can only state that this is an acceptable risk because the potential benefits are large and the dangers purely hypothetical. Needless to say, countries will differ concerning what they regard as an acceptable risk. Some ban DDT and saccharin; others consider that the benefits derived from these substances justify their use.

The American people may decide that the safest course is a moratorium on DNA recombinant research, or regulations limiting it to a few institutions. Such policies would paralyze research in the United States but would not affect it in other countries.

DNA recombinant research will go carried out almost anywhere because it requires only simple equipment, unless put into a cumbersome straitjacket by unreasonable safety measures. DNA recombinant research will go on in many places for several reasons. It enables even poor countries to engage in the most sophisticated field of biological science. It promises practical applications in medicine, agriculture and industry. It is one of the most exciting areas of knowledge, with large philosophical and scientific implications for the understanding of life.

Excerpted from "Recombinant DNA: Fact and Fiction," Cohen, Stanley N., Science Vol. 195, pp. 654-657, 18 February 1977. Copyright © 1977 by the American Association for the Advancement of Science.

Much has been made of the fact that, even if a particular recombinant DNA molecule shows no evidence of being hazardous at the present time, we are unable to say for certain that it will not devastate our planet some years hence. Of course this view is correct; similarly, we are unable to say for certain that the vaccines we are administering to millions of children do not contain agents that will produce contagious cancer some years hence, we are unable to say for certain that a virulent virus will not be brought to the United States next winter by a traveler from abroad, causing a nationwide fatal epidemic of a hitherto unknown disease—and we are unable to say for certain that novel hybrid plants being bred around the world will not suddenly become weeds that will overcome our major food crops and cause worldwide famine.

The statement that potential hazards could result from certain experiments involving recombinant DNA techniques is akin to the statement that a vaccine injected today into millions of people *could* lead to infectious cancer in 20 years, a pandemic caused by a traveler-borne virus *could* devastate the United States, or a new plant species *could* uncontrollably destroy the world's food supply. We have no reason to expect that any of these things will happen, but we are unable to say for certain that they will not happen. Similarly, we are unable to guarantee that any of man's efforts to influence the earth's weather, explore space, modify crops, or cure disease will not carry with them the seeds for the ultimate destruction of civilization. Can we in fact point to one major area of human activity where one can say *for certain* that there is zero risk? Potentially, we could respond to such risks by taking measures such as prohibiting foreign travel to reduce the hazard of deadly virus importation and stopping experimentation with hybrid plants. It is possible to develop plausible "scare scenarios" involving virtually any activity or process, and these would have as much (or as little) basis in fact as most of the scenarios involving recombinant DNA. But we must distinguish fear of the unknown from fear that has some basis in fact; this appears to be the crux of the controversy surrounding recombinant DNA.

Unfortunately, the public has been led to believe that the biohazards described in various scenarios are likely or probable outcomes of recombinant DNA research. "If the scientists themselves are concerned enough to raise the issue," goes the fiction, "the problem is probably much worse than anyone will admit." However, the simple fact is that there is no evidence that a bacterium carrying any recombinant DNA molecule poses a hazard beyond the hazard that can be anticipated from the known properties of the components of the recombinant. And experiments involving genes that produce toxic substances or pose other known hazards are prohibited.

Editorial by Richard Goldstein, Ph.D. Copyright © 1977 by the New England Journal of Medicine, Vol. 296, pp. 1226-8, May 26, 1977. Reprinted by permission.

PUBLIC-HEALTH POLICY AND RECOMBINANT DNA

THE issue of recombinant DNA research (i.e., gene manipulation) has been discussed widely in the public press and scientific journals. The controversy has been the focus of numerous meetings sponsored by the National Institutes of Health and most recently by the National Academy of Sciences. It is regrettable, therefore, that the report in this issue of the *Journal* by C. Cohen completely ignores the central questions in the debate. It dismisses potential hazards associated with the technology while attempting to lay down a priori principles on which to make judgments of little relevance. The report is an entertaining intellectual exercise with no bearing on the facts involved. Worse yet, Cohen demonatrates a lack of understanding of science and its history, and particularly of the specifics of recombinant DNA research. The real issue — a determination of sound public-health policy taking into account both potential benefits and hazards — is not addressed. Rather, the bulk of his thesis revolves around questions of complete prohibition, a position not shared even by critics of the research whose views, in my opinion, he so distorts. Cohen seems unaware of the difference between reasonable constraint, a view espoused to varying degrees by most of those on both sides of the debate, and absolute prohibition. This view sets back the controversy instead of constructively helping to define the issues. It is a disservice to the proponents of the research who have struggled so diligently at self-regulation.

Recombinant DNA technology makes it possible to remove DNA from any organism, to chop the DNA enzymatically into smaller sequences of genes, to splice these genes into an appropriate vector (usually a virus or plasmid), and then to use the vector to infect an animal, plant or bacterial cell of choice. The end result then is the insertion of the foreign genes of interest into a new host. This revolutionary technic permits genetic information to cross species barriers readily. It has allowed genes derived from animal cells and their viruses to be implanted into bacteria, and bacterial genes to be inserted into animal cells. The usual "time gap" (a potential safety valve) between a fundamental discovery in basic science and its application as a technology disappeared in record time with recombinant DNA studies. With its capacity to rearrange the genetic heritage of millions of years of evolution, this technology appears to offer a power to transform living organisms comparable to the power of nuclear fission to transform matter.

Recombinant DNA technology will have profound effects on biomedical sciences, industry and society at large. There is indeed the potential for benefits of large magnitude. With regularity, bacteria are now being implanted with nonbacterial genes. These genes are then reproduced as the genetically modified bacteria reproduce, thereby making it possible to obtain the gene products for which the implanted genes code. So programmed, bacteria are envisioned as factories for the production of hormones, antibiotics, antibodies and enzymes of potential use in medicine and industry, as well as other gene products needed for basic research. So engineered, the normal control systems regulating bacterial growth and function may be circumvented.

Recombinant DNA technology is now in widespread use in basic-research laboratories, with the number of such experiments constantly increasing. A not unreasonable estimate is that 10^{15} to 10^{16} recombinant organisms per year will be produced in the laboratories and that this number will be substantially increased by industrial involvement.[1] Like the benefits of nuclear-power plants, the benefits of recombinant DNA research are likely to come with mixed peril. The National Institutes of Health and other proponents of the technology are to be lauded for their efforts in struggling with this dilemma and the resultant development of guidelines for future studies. In some instances these guidelines seem particularly effective when dealing with the more obvious concerns of public-health nature by prohibiting overtly dangerous classes of experiments — for example, the cloning of recombinant DNA's from known oncogenic viruses or pathogens.[2]

The real shortcoming of the guidelines is that they do not give equal importance to potential problems developing from less obvious situations than the known pathogens mentioned above. The enormous complexity of micro-organisms and ignorance of their intricately complicated ecology make it impossible to predict with certainty the end effect of any transplant of large numbers of genes into the micro-organism of choice, *Escherichia coli* K-12, or a further enfeebled genetically modified strain derived from K-12. *Esch. coli*

is indigenous to human beings and all warm-blooded animals, is carried by many other organisms such as insects and fish, and can be found throughout the biosphere in soil and water. This microbe can normally exchange genetic information with 40 other species of micro-organisms, and though the likelihood of exchange from the genetically enfeebled strain has been markedly reduced, it still may occur. The vast sea of micro-organisms in the environment makes events whose probability is the reciprocal of Avogadro's number (i.e., $1/10^{23}$) not impossible.*

Many experiments being carried out employ "shotgun" technics where complete chromosomes of undefined gene sequence are split into many segments followed by insertion of these unknown genes into *Esch. coli* with potentially unpredictable results. Every implanted gene becomes a new hypothesis, and there will be tens of thousands of such new hypotheses inserted. This is the "Catch-22" dilemma associated with the technology. Could we inadvertently, via unexpected transfer of genetic information from the enfeebled host strain to one of the estimated 10^{13} to 10^{14} healthy *Esch. coli* organisms in the human bowel, create a novel self-replicating pathogen? Do we know for certain the effect in human beings or any other creature of the biosphere, of a novel micro-organism producing a powerful insulin-like hormonal activity? Cloning of the insulin gene is but one of many experiments now in progress, any of which could have potential toxic or autoimmune effects. What effect might such a micro-organism have on other varieties of enfeebled hosts — the human patients who are on antibiotics therapy, who are immunosuppressed or who are otherwise debilitated by disease. In some cases these patients reside in hospitals that also include recombinant DNA research laboratories. We do not as yet know the answers to the above questions. These questions should be addressed, and their answers and solutions framed in a new and badly needed public-health policy.

That the NIH is providing millions of dollars for construction of containment facilities for some of this research is to its credit. Yet the "Fort Detrick Experience"[3] and the records of the Center for Disease Control make it painfully clear that absolute containment of micro-organisms is impossible — even in laboratories where workers are exceedingly well trained and experienced in working with pathogenic organisms. Human frailty will guarantee mistakes. What, then, can be expected from numerous new laboratories recently involved in recombinant DNA research whose personnel have at best had a three-day course on containment procedures? Here, we have the potential for a unique form of pollution, biologic pollution, and it will be distinguished by its irreversibility. In contrast to nuclear-power plants, which can be closed down, micro-organisms will reproduce and perpetuate themselves. They cannot be recalled. Unlike the defined pathogens studied at Fort Detrick, their escape cannot be monitored for.

The great deal of time and effort put into self-regulation, and the development of biologic and physical containment systems by the proponents of the technology, is unprecedented. They have set an example of genuine concern for human well-being and the biosphere. But they have not faced containment of their own exponentially expanding activities, which may be an equally irreversible route to ecologic disaster at the rate at which the research activities are expanding. In reality, we have all become experimental subjects for recombinant DNA experiments. Yet none of us have signed an informed-consent statement.

A risk-assessment panel has recently been convened by the NIH to determine the risks involved and where information is lacking to propose experiments to obtain the information. This is a proper and important step in terms of public-health considerations. The burden of proof or lack of hazards should rest neither on the opponents nor on the proponents of the research. Their role should simply be to distill and define the issues for the remainder of the biomedical community and the public at large. The go-ahead signal should be the result of a public-health decision-making process, developed by decision-making bodies democratically appointed and representative of a rich diversity of ethical and scientific points of view.

And in the interim? I believe that the research should proceed, but on a more limited basis, perhaps only at regional facilities of high containment staffed by experienced and well trained personnel. A host bacterium less ubiquitous than *Esch. coli* also seems a wiser choice. Such a temporary slowdown would have no effect on freedom of inquiry. Rather, it would effect freedom of manufacture — the manufacture of novel self-replicating micro-organisms. A miscalculation on our part as scientists would have far more damaging effects than a temporary slowdown on science as we know it in terms of public outrage and mistrust.

The societal benefits that may well arise from gene transplantation studies will be valuable whether they come in 20 years versus 25, or 50 years versus 55. These benefits and the sheer excitement and beauty of

*Curtis R. Potential biohazards of recombinant DNA molecules (unpublished memorandum to Paul Berg et al.) August 6, 1974

scientific endeavor will endure for centuries thereafter. For five or 10 years now, a slow, thoughtful approach to limiting hazards makes sense in terms of progressive public-health policy.

Harvard Medical School
Boston, MA 02115 RICHARD GOLDSTEIN, PH.D.

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2. Beneficial Impacts of Recombinant DNA Research

Section IV-C-2 of this EIS describes the various anticipated benefits of recombinant DNA research. As with the possible hazards, many of the proposed benefits are hypothetical. Assessment of the likelihood that they will be realized will depend on information acquired from future experimentation. For example, assessment of the category of anticipated benefits that depends on the synthesis of eukaryote proteins in prokaryote cells [see Section IV-C-1-b-(1) of this EIS] awaits additional data on the expression of the foreign genes. Should these benefits be realized, it may be expected that the cost of manufacturing certain clinically important proteins can be markedly decreased. Other clinically important proteins that are either in short supply (e.g., human growth hormone) or unobtainable by existing techniques may be made readily available. Innovative approaches to immunization against infectious diseases can also be expected.

Some of the indicated benefits appear certain. These are the benefits to be derived from an increased understanding of both basic biological processes and the mechanisms underlying a variety of disease states.

Application of the restrictions imposed by the Guidelines will retard progress toward the realization of the possible benefits. In addition to the prohibitions on certain experiments, there are many permissible experiments that will need to be postponed until the requirements in the Guidelines can be met. An increase in the number of P3 facilities will require adequate funds, extensive planning, and installation. P4 facilities are limited in number. Experiments that require host-vector systems with demonstrably limited ability to survive in natural environments must await development of such systems, their testing, and finally their recommendation for approval by the NIH Recombinant Advisory Committee and their certification by the NIH Director. Time will also be required for the various review processes.

As of this writing, certain EK2 host-vector systems have been recommended for approval and others have been recommended and certified (Appendix H, part III). Information on these EK2 host-vector systems is available from the NIH Office of Recombinant DNA Activities.

Appendix H, part I, contains criteria for construction of EK2 systems using lambda phage vectors, as recommended by the Working Group on Safer Hosts and Vectors: Lambda Phage Systems and adopted by the Recombinant Advisory Committee at their meeting on January 15, 1977.

Appendix H, part II, contains instructions to investigators concerning data to be submitted on host-plasmid systems proposed for EK2 certification, as adopted by the Recombinant Advisory Committee on March 21, 1977.

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VIII.
RESPONSES TO COMMENTS ON THE DRAFT EIS

Introduction

Notification of the availability of the Draft Environmental Impact Statement appeared in the Federal Register of September 2, 1976, and was announced in a press release and in the NIH Guide to Grants and Contracts. The Draft EIS was published in the Federal Register of September 9. A partial list of the 2,000 persons and organizations to whom it was sent for review is given in Appendix L. Thirty-eight letters of response were received and are reproduced in their entirety in Appendix K.

Chapter VIII contains the NIH analysis of these comments and the responses to them. While every comment is not addressed separately, all of the material issues raised in the comments are covered.

An index at the beginning of Appendix K identifies each commentator, shows the document number of each, and indicates the sections in Chapter VIII where responses to the commentator are given. In addition, the text of VIII identifies at least one commentator for each response (document numbers in brackets).* On the basis of the considerations represented in VIII, the text of the EIS has been modified in numerous places.

The organization of the Draft EIS, the first seven chapters of the Final EIS, and Chapter VIII of the Final EIS are all identical.

It should be noted that the comments did not contain any scientific evidence relative to risk-assessment which had not already been considered during construction of the NIH Guidelines.

Although all comments were requested for submission by October 18, 1976, extensions were given to several commentators at their request. The receipt of formal comments was continued until December 15.

A number of the comments in response to the Draft EIS were actually addressed to the Guidelines. These were forwarded to the Recombinant Advisory Committee and are being considered as changes in the Guidelines are contemplated.

From here on, this chapter follows the organization of the Draft Environmental Impact Statement and the preceding chapters.

*Reference numbers are in parentheses.

I. FOREWORD

In this section of the Draft EIS, the development of the "NIH Guidelines for Research Involving Recombinant DNA Molecules" was discussed. Some commentators considered the public role in that development inadequate [12, 26, 30]. The National Institutes of Health recognizes its responsibility to ensure opportunity for public review and participation. All meetings of the Recombinant Advisory Committee were advertised in the Federal Register and open to the public, and as previously noted, a public hearing on the proposed Guidelines was held at NIH in February 1976. The Guidelines were revised on the basis of that public response. They were then issued, on June 23, and were published in the Federal Register on July 7 to provide further opportunity for comment. Accompanying the NIH Guidelines was the "Decision of the NIH Director" addressing the issues that public commentators had raised at the February hearing and in subsequent correspondence.

The publication of the Draft EIS in the Federal Register provided yet another means to obtain public participation. The Guidelines also provide for public review of the conduct of research in that they recommend broad representation on all institutional biohazards committees that oversee the research. The NIH scientific committee responsible for developing and reviewing the Guidelines (Recombinant Advisory Committee) holds open meetings, and its actions, in turn, are reviewed and monitored by a public advisory group to the NIH Director.

A number of commentators observed that the Guidelines were released prior to completion of the environmental impact assessment. They asserted that this action was in technical violation of NEPA [12,20,26,30]. As explained here (and in the Draft EIS), however, the NIH maintains that the public interest required immediate issuance of the Guidelines to protect the safety of persons conducting this work and of the general public.

A number of commentators did not agree that the issuance of the Guidelines was a "major Federal action" as defined under NEPA [20,26], but suggested instead that the major Federal action was NIH's funding of research. In fact, none of the NIH individual grants or contracts involving recombinant DNA research supports such research per se. Rather, this research represents merely one among many techniques used under these grants and contracts for basic biomedical research aimed at ultimately improving the Nation's health.

One commentator held that the Draft EIS, instead of being limited to NIH, should have been a statement drafted by NIH as a lead agency for all Federal agencies [20]. This suggestion erroneously assumes that there is a broad government recombinant DNA research program. At present, among Federal agencies, only the National Science Foundation and the Department of Agriculture, in addition to NIH, have grants or contracts in recombinant DNA research. As of August 1977 NSF was supporting 65 projects that included recombinant DNA research in whole or in part. The Department of Agriculture, together with the Cooperative State Research Service, was supporting approximately 50 such projects, and Agriculture's Research Service had four.

Although no other Federal agencies are supporting recombinant DNA research, all those represented on the Federal Interagency Committee (see Appendix I) have endorsed the NIH Guidelines.

The NIH Environmental Impact Statement may serve, if appropriate, for other agencies. All Federal research agencies were sent copies of the Draft EIS for comment and will be sent a copy of this Final EIS.

II. AUTHORITY

No significant issues were raised concerning this section of the Draft EIS.

III. OBJECTIVE OF THE NIH ACTION

See discussion of the "major Federal action" in Section I of this appendix.

IV. BACKGROUND

A number of issues were raised by commentators in this section of the Draft EIS.

A. Description of the Recombinant DNA Experimental Process

In response to the requests by some commentators, additional descriptions and references concerning the natural biological processes of recombination and transformation have been added to this Section [20,26]. New material was included to make clear that recombinant DNA technology is a tool that can be applied to diverse areas of biological research.

It was pointed out by two groups of commentators that the Draft EIS did not discuss techniques for the isolation of large amounts of DNA sequences corresponding to genes by techniques other than recombinant DNA [20, 30]. While it is true, as pointed out by the commentators, that certain DNA sequences of sufficient purity have been isolated in useful amounts by classical biochemical techniques, the methods are relatively specific and yield only small amounts of the desired fragments, thus making demonstration of purity questionable in many instances. It is relevant to the consideration given to this comment that, with one exception--Dr. John Sedat's testimony in the transcript of a public hearing on recombinant DNA, February 9-10, 1976, at the NIH (1)--no other commentator on the Draft EIS, and no other participant in the 3-year discussion of recombinant DNA experiments, has seriously proposed that classical (non-recombinant) techniques could be usefully employed for the large-scale isolation of highly purified genes.

The examples cited by these commentators [30 (2, 3)] refer to the isolation of DNA segments corresponding to the genes for mammalian globin (the protein portion of hemoglobin). The techniques are useful only when the messenger RNA for the particular gene itself can be isolated intact and in highly purified form in substantial quantities. Such procedures are applicable only to a small set of proteins made in highly specialized situations within organisms. Indeed, the DNA preparations described [30 (2, 3)] were specifically prepared for insertion into vectors, and subsequently host cells, by recombinant DNA procedures, in order to obtain useful quantities of the DNAs in question. Furthermore, these techniques, even ideally, are of limited value, since it is impossible to isolate by classical procedures those portions of the DNA contiguous to the gene which may play essential roles in gene expression and regulation.

Insight into the relative advantages of classical and recombinant DNA techniques for the study of gene structure can be obtained by comparing the application of the two approaches to the study of histone genes. In this instance progress was again dependent on the ability to isolate relatively pure messenger RNA as well as on the special advantage obtained when the genes themselves occur many times in the DNA of the organism. While substantial information has been obtained by classical procedures (2, 3), isolation of the necessary DNA in sufficient quantity and purity was very difficult. These problems were effectively circumvented by recombinant DNA technology (4, 5). The group utilizing the classical techniques (2) has recently stated that they are now isolating the histone genes by recombinant DNA methodology (2).

For problems involving genes that are not repeated many times, or those for which isolation of the corresponding messenger RNA is not feasible, recombinant DNA techniques offer the only approach to gene isolation.

For these reasons the alternatives raised by these commentators are not specifically included in the text of the EIS.

B. Events Leading to the Development of Guidelines

Several commentators considered inadequate the public participation in the development of the NIH Guidelines, as noted in Section I above [12,26,30]. The NIH has been attempting, and will continue to attempt in the future, to achieve the greatest possible public participation and review with respect to the problems involved in research involving recombinant DNA molecules. Specific suggestions are welcome as to how the NIH might be able to engage more fully the attention and participation of the general public.

Some of the comments suggested that the Draft EIS was not clear regarding the relation between experiments included in the 1974 voluntary partial moratorium and the recommendations in the Asilomar report and the NIH Guidelines [26,27]. The quotation from the letter of the NAS committee (July 7, 1974) calling for the partial moratorium is now more extensive, and the historical relations are more explicitly described in this Section. The experiments currently prohibited by the NIH Guidelines are now described in some detail (see EIS Section VI), and a comparison between these prohibitions and those of the 1974 moratorium can be made. At no time has there been an official moratorium on all recombinant DNA experiments. Certain experiments proceeded during the period 1974 through publication of the NIH Guidelines in June of 1976 (see EIS IV-B).

Some commentators urged that the nature and scope of recombinant DNA research funded by the NIH and other Federal agencies be described [10,20,26]. The Draft EIS did not include specific information on the number or location of laboratories currently engaged in recombinant DNA work. Comments stated that such information was important for realistic assessment of the impact of the Guidelines. Such information is now provided in Appendix G of the EIS, and the text so states.

In addition, material has been added to the text which highlights some important aspects of the Guidelines, including the functions of Institutional Biohazards Committees and Memoranda of Understanding and Agreement (MUA).

C. Description of Issues Raised by Recombinant DNA Research

1. Possible Hazardous Situations

Several of those commenting on the Draft EIS pointed out the lack of sufficient discussion of possible evolutionary consequences of the accidental dispersion of organisms containing recombinant DNA [2, 20, 25, 26, 29, 30]. The omission of this matter from the Draft EIS was based on several considerations, including the almost total lack of relevant scientific facts, the highly controversial nature of modern evolutionary theory, the consequent inability to impose a theoretical framework on the issue, and difficulties in analyzing the arguments of those who have expressed serious concern with this matter--difficulties that arise from the very general way in which the problem has been stated. While most of the issues discussed and evaluated in the EIS involve speculations and judgments, none is as conjectural as the question of evolutionary consequences.

For these reasons it has been extremely difficult to deal with this issue in the formulation of policy. The text of the EIS, however, now includes a more complete discussion of the survival of organisms containing recombinant DNA molecules. In an effort to respond to the comments, the question is further described in Section VII-B-1-b of the EIS, and references are given to published discussions.

2. Expected Benefits of Recombinant DNA Research

The portion of this Section dealing with the use of recombinant DNA techniques to elucidate basic biological processes has been expanded. This should help readers to understand the uses and value of this new tool. Throughout the Section, additional references to the scientific literature and other documentation have been incorporated.

3. Long-Range Implications

A commentator suggested that it be made clear in this Section that conventional plant and animal breeding approaches do not include recombinant DNA experiments that are governed by the NIH Guidelines [37]. The Draft EIS pointed out that recombinant DNA research methods may offer new opportunities for specificity and accuracy in animal breeding. However, a statement has been included to distinguish animal and plant breeding from recombinant DNA laboratory research.

Two commentators suggested more discussion on the ethics of "genetic engineering."[20, 35] In the Draft EIS it was noted that the use of recombinant DNA research techniques for the possible correction or amelioration of human genetic defects raises complex and difficult problems requiring serious public debate and consideration. Recombinant DNA research, however, is not directed to the altering of human genes, and its potential for doing so in the near future is questionable.

Since the publication of the draft EIS, a number of developments have occurred in Congress as well as the Department of Health, Education, and Welfare concerning studies to be undertaken to ensure thorough review of the social, ethical, and legal implications of genetic techniques such as recombinant DNA to alter the genetic character of man and other forms of life. Further discussion of those developments is included in the text of the EIS.

4. Possible Deliberate Misuse

Some commentators considered this section in the Draft EIS too brief and sanguine, for they believed that recombinant DNA technology might yield intentionally hazardous agents [2, 11, 17, 30, 32, 33]. It is not certain that recombinant DNA research techniques could produce hazardous organisms. Any discussion of potential misuse by saboteurs would be entirely speculative. Further efforts would, of course, be necessary if future scientific or safety information shows that the problem is less hypothetical. This Section of the EIS has been expanded by inclusion of remarks by Ambassador Joseph Martin, Jr., stating that the use of recombinant DNA for biological warfare is prohibited by the Biological Weapons Convention.

V. DESCRIPTION OF THE ACTION

Section V of the EIS has been substantially revised and expanded. Not only have four new subsections been added, but an attempt has been made to document more fully the rationale underlying the Guidelines, to describe better their prohibitions and requirements, and to explain the application of physical and biological containment. The text dealing with administrative framework relative to compliance with the Guidelines has been augmented.

A. General Application of the Guidelines

This Section of the EIS is devoted to a description of the science and safety standards of the NIH Guidelines and their implementation by the NIH, local institutions, and investigators. A number of commentators directed their remarks to roles and responsibilities for safety as defined in the Guidelines.

One commentator noted that the Draft EIS gave little attention to research specifically dealing with recombinant DNA from plant sources and the possible environmental consequences of their escape [19]. The analysis of potential hazards in the EIS is given in a general way that is equally applicable to persons, animals, and plants. This is now pointed out explicitly in Section IV-C and VII-B-1 of the EIS. Specific requirements for work with plants are included in the Guidelines. Containment requirements for the insertion of plant DNA into E. coli host-vector systems are given in Section III-B-2 of the Guidelines, and containment requirements for the use of plant host-vector systems are given in Section III-B-4-b of the Guidelines.

Some commentators on the Guidelines expressed reservations about the adequacy of either physical or biological containment [12,25,29]. Section V has been revised to explain more fully how these containment measures operate, both individually and in combination. The exact requirements for each level of physical and biological containment were carefully considered during the development of the Guidelines, and are being reexamined during consideration of the revision of the Guidelines which is already under way. The NIH has concluded that the greatest level of safety can be obtained by the complementary use of both physical and biological containment. It is precisely because neither alone is infallible that the complementary use of the two, for every experiment, significantly decreases the likelihood that potentially hazardous organisms will escape and cause significant harm.

B. Physical Containment Requirements

With respect to physical containment safeguards, one commentator recommended that the EIS should specifically refer to the requirement in the Guidelines for an insect and rodent control program for all levels of physical containment [11]. Others suggested that the EIS refer to the requirement in the Guidelines that, at all levels of physical containment, liquid and solid waste contaminated with recombinant DNA material must be decontaminated before disposal [22,29]. The EIS now contains the following statement: "Please note that Appendix D to the Guidelines (see Appendix D to this EIS) provides detailed supplementary information on physical containment. Among many other items are specifics on care and use of laboratory animals, requirements for an insect and rodent control program at all containment levels, and guidance on decontamination and disposal of liquid and solid waste."

For clarity, the text in this section has been expanded with examples of the types of non-recombinant DNA work which are conducted at each of the four levels of physical containment (P1-P4).

C. Biological Containment Requirements

Some readers of the Draft EIS noted that the term biological containment was not used in the statement, while it has been used extensively in the Guidelines and in public discussion of recombinant DNA issues. The term was omitted from the Draft EIS on grounds that it might be confusing to lay readers. Confusion can arise because "biological containment" does not, in fact, contain the organisms in the sense that their dispersion from the laboratory is controlled. Rather, the term is used to describe organisms that, were they to escape the laboratory, would have only a slight probability of growing in natural environments. The concept of biological containment is discussed in detail in Sections II and III of the Guidelines.

In addition to explaining the concept of biological containment, this section describes the properties of the E. coli K-12 host-vector systems. (See also Section VI-C-3-b.)

D. Combination of Physical and Biological Containment in Practice

Within the EIS the concept of biological containment is now explicitly stated. It is also implied in the framework for assessment for danger given in Section V-D. Extensive material has been added to this section explaining the Guidelines' standards as applied to particular types of experiment and presenting examples of containment systems. Items (4), (5), (7), and (8) in the list beginning "The criteria for assessing the possibility for danger are:" concern the ability of the vector or the host cell to survive in natural environments or to transmit recombined genes to other organisms that might be encountered in natural environments. Since the possibility for danger will increase with the degree to which these items in the list increase (as indicated in this Section), the use of host-vector systems chosen or designed to minimize these possibilities (such as EK1, EK2, EK3, or defective polyoma and SV40 viruses) decreases the potential for hazard in a given experiment.

An explanation for the requirement of higher containment levels for experiments involving recombinant DNA from higher organisms (e.g., primates) appears in this section under the subhead "Examples of Containment Systems" [26].

For the convenience of readers for whom the Guidelines themselves are too technical, a review of these concepts and of the Guidelines themselves can be found in an added Appendix E to the EIS.

E. Compliance with the Guidelines

A number of commentators recommended that there be a more complete discussion on NIH implementation of the Guidelines [20,26,29]. Appendix D to Appendix C of the EIS now contains memorandums from Dr. William Gartland, Director of the Office of Recombinant DNA Activities (ORDA), to all NIH grantee institutions and contractors which provide the structure for the implementation. The text of the EIS has been expanded to explain more fully compliance procedures and responsibility.

Some commentators suggested that membership of Institutional Biohazards Committees should include specialists in population dynamics, ecology, and other disciplines [10,20,29]. The Guidelines recommend that committees have representatives with the competence to determine the acceptability of committee findings in terms of applicable laws, regulations, standards of practices, community attitudes, and health and environmental considerations. It is also recommended that representatives from disciplines relevant to recombinant DNA technology, biological safety, and engineering serve on the committees. Inclusion of specialists in ecology and population dynamics would be within the intent of the Guidelines for committee service.

Some commentators were concerned that the Draft EIS did not emphasize relevant safety training for laboratory personnel [3,20]. The Guidelines, however, specifically require the principal investigator (with the overview of the Institutional Biohazards Committee) to ensure appropriate safety training of all personnel prior to the conduct of the experiment. Further, the Biohazards Committee must review and certify that the investigator does fulfill this responsibility, both at the time of initial application for research support and annually thereafter.

The commentators generally noted the limited scope of the NIH Guidelines as not reaching research in the rest of the public sector or in the private sector [14,17,20,26,29]. As described below, however, (see Section VI-E) the Federal Interagency Committee on Recombinant DNA Research recommended that legislation be developed to regulate all recombinant DNA activities, and an Administration bill based on those recommendations was submitted to the Congress. Legislation is pending.

Some commentators observed that the NIH sanction of terminating funds for violation of the Guidelines may not be the best approach [14,20,29]. More limited actions might be taken; for example, higher containment levels or other safety steps might be required. The NIH will consider these suggestions in developing policy. The pending legislation imposes more stringent measures than those available to NIH alone.

Several commentators were concerned about the adequacy of the inspection, certification, and surveillance processes to insure compliance with the NIH Guidelines [20,29]. One thought that NIH staff should perform site inspections of P3 as well as P4 facilities and recommended that NIH consider cooperative Federal-State inspection capabilities [20].

Many aspects of the "Roles and Responsibilities" Section (IV) of the NIH Guidelines address the issues relating to inspection, certification, and surveillance. These roles and responsibilities are shared among the principal investigator, the institution, and NIH. The investigator is responsible for supervising safety performance at the bench level, and for reporting to the institution and to NIH (a) new information bearing on Guidelines, hazards, or safety procedures, (b) evidence of serious and extended illness among staff or serious accidental exposure to staff or environment, and (c) any problems pertaining to operation and implementation of biological- and physical-containment requirements. The NIH Guidelines require the institution to see that the responsibilities of the principal investigator are carried out. They also require the institution to establish an Institutional Biohazards Committee which, among other responsibilities, shall certify that facilities, procedures, practices, training, and expertise of involved personnel are adequate for the safe conduct of NIH-sponsored research and that they comply with the NIH Guidelines.

Because of the different administrative schemes used by institutions engaged in recombinant DNA research, it is impossible to make specific rules guaranteeing the best method to provide institutional certification and surveillance of this work. NIH, however, is developing general guidelines for Institutional Biohazards Committees which will recommend several approaches for providing certification and surveillance. In this regard, recognition will be given to the significant role that environmental health and safety professionals can perform in providing support to the researcher and informing management on issues relating to compliance.

NIH does not plan to routinely inspect P3 facilities. The safety aspects of such facilities can be adequately certified by institutional authorities and verified by review of the research application and supporting documentation. NIH will, however, conduct site inspection of all P4 facilities in which NIH-supported recombinant DNA research is to be performed. Inspection protocols and recommendations concerning frequency of site inspections are being developed by NIH staff.

NIH is not considering the delegation to State or local authorities of on-site inspection of P4 facilities in which NIH-supported recombinant DNA research is to be conducted.

Several commentators called for more attention to medical surveillance and epidemiological measures in the event of possible infection of the laboratory worker or contamination of the environment [10,20,29, 32]. Others recommended a more detailed discussion in the EIS on measures to ensure appropriate safety training for laboratory personnel [20]. The Guidelines (Section IV) specify in detail the responsibilities of the investigator and the institution to ensure appropriate safety training and monitoring to minimize exposure to hazards.

Several commentators suggested that local and State authorities must be involved in the process at the local level [20,32]. As noted before, the Guidelines recommend that the Institutional Biohazards Committees have representatives familiar with applicable laws, regulations, standards of practice, community attitudes, and health and environmental considerations. The local health officer, for example, or the State health representative could be kept informed by serving on the Biohazards Committee.

And in addition, legislation pending in both House and Senate would allow States to act in this area under certain conditions.

Several commentators inquired about plans to generate new information relative to safety in research involving recombinant DNA molecules [19,20,29,30]. New efforts relevant to this comment have been initiated in three broad areas: (a) biohazard assessment, (b) development of safer host-vector systems, and (c) training.

Biohazard Assessment. An NIH intramural program has been initiated to assess the nature of biohazards that may be associated with the production of recombinant DNA molecules and their subsequent amplification in both prokaryotic and eukaryotic cells. The ability of recombinant DNA molecules, and vectors and hosts containing such molecules, to survive under laboratory and normal environmental conditions will be evaluated under an extramural contract program that has been initiated. A contract effort has also been initiated to quantify the exposure potential that may be created by a variety of experimental procedures used in research involving recombinant DNA molecules and by simulated accident situations. Results of these efforts will make it possible to establish more precise estimates of potential hazards and to select the most appropriate methods of physical containment.

Development of Safer Host-Vector Systems. An extramural contract program has been initiated to develop safer host-vector systems and to verify their genotypic constitution and phenotypic traits. The program is administered by the Research Resources Branch of the National Institute of Allergy and Infectious Diseases. The ability of these developed and verified systems to survive in laboratory and natural environments will also be determined. This effort is being undertaken to insure the availability of safer host-vector systems for cloning recombinant DNA molecules.

Training. Formal training programs in biological safety are being intensified. They are presented in geographical locations near centers of recombinant DNA research. The biohazard assessment information being developed under our intramural and extramural programs is rapidly incorporated into this training effort.

It has been recognized that research groups are likely to be more knowledgeable in the biology of the recombinant DNA system employed than in biohazard control measures. The NIH has undertaken a number of other initiatives since release of the Guidelines for developing courses in laboratory safety and training personnel in handling biohazards. For scientists who will become engaged in such research, the NIH has initiated a training course on basic principles of biohazard control. The purpose is to make researchers aware of the expertise that is required to control biohazards successfully and to provide guidance in gaining this expertise. The course involves both lectures and workshops. Orientation is toward physical containment methods appropriate for P1, P2, and P3 level biohazards. A comprehensive course manual providing an extensive reference of biohazard materials is given to each attendee. There is to be no provision for certifying proficiency.

Other training initiatives are in progress. An instructors' manual is being prepared so that the basic biohazards course may be easily adopted and presented by institutions that may wish to establish formal training programs in biohazard control. The NIH is currently developing training opportunities designed to prepare researchers to employ P4 physical containment practices proficiently. Also, several NIH components continue to produce training aids, pamphlets, and materials on the broad subject area of biohazard control. These efforts will contribute to the training of scientists throughout the world in biohazard control techniques.

The NIH will also conduct site inspections of all P4 facilities in which its grantees and contractors will engage in recombinant DNA research. This inspection program will examine the training provided both to the research staff and to personnel who are responsible for the operation of P4 facilities.

VI. DESCRIPTION OF ALTERNATIVES

A. No action.

There were no specific comments directed to this suggested alternative. The text of the EIS, however, has been revised and updated.

B. NIH Prohibition of Funding of All Experiments with Recombinant DNA

Here too, the text has been revised to reflect current facts.

Commentators noted that this alternative is properly one for the entire Federal Government and not just NIH [18,20,26]. Because the Environmental Impact Statement addresses the NIH Guidelines, this alternative relates only to the NIH role. However, the issues raised can apply to all Federal research agencies or departments that may support or conduct this research, and all have endorsed the NIH Guidelines standards. Some commentators were not persuaded by the statement that U.S. leadership in biological research would be threatened by prohibition of all recombinant DNA experiments [20,26]. It should be noted that recombinant DNA research has been reviewed by many national and international bodies, all of whom have recommended that such research should continue under appropriate safeguards. Its prohibition in the United States would severely impair our research leadership.

C. Development of Different Guidelines

In the text of this section of the EIS, subsections VI-C-2 and -3 of the Draft have been consolidated into one subsection, VI-C-2. Thus, subsection VI-C-4 of the Draft is now VI-C-3.

1. Level of Physical Containment

Several commentators expressed dissatisfaction with the NIH Guidelines. For example, it was pointed out that the Guidelines do not explicitly require assurances that physical-containment equipment be properly designed, installed, and certified as to proper use, or about the Guidelines' risk-classification system. These comments were referred to the NIH Recombinant Advisory Committee, an action deemed appropriate because such comments related more to the contents of the Guidelines themselves than to the EIS. Moreover, the NIH Recombinant Advisory Committee was in the process of recommending changes in the Guidelines to the NIH Director, and would be able to take these additional issues into consideration. The basis of the current Guidelines is explained throughout Sections IV through VII of this EIS.

2. The Recommendation That All Permissible Recombinant DNA Experiments Be Conducted in National Facilities

A number of commentators addressed their remarks to alternatives in which all recombinant DNA research would be conducted in a single or a few national facilities [12, 27, 30], or all such work would be limited to P3 or P4 facilities until more is known about the new techniques [20, 24, 26, 29]. Because these suggestions represent variations of the same theme--namely, the restriction of all DNA research activities to certain facilities--they raise basically the same issues, and the EIS has been revised to discuss these alternatives as a whole. Thus, where the Draft EIS had contained two separate sections ("establish a few national P3 facilities" and "conduct all recombinant DNA experiments in P4 facilities"), the revised EIS takes up this subject in a single section, VI-C-2. The provision of national facilities for conducting some of the research requiring P4-level containment is feasible and is being considered by the NIH.

3. Experiments Prohibited at This Time

A number of commentators would prohibit certain classes of experiments that are now permissible under the Guidelines [12, 34]. Several expressed concern over the advisability of permitting recombinant DNA experiments using E. coli host-vector systems [3, 20, 25, 30, 36]. As a result, Section VI-C-3-b of the EIS has been expanded to describe the situation in more detail [7]. Several useful technical references are given. These questions are also discussed in some detail in the Guidelines themselves (Section III-A).

A new appendix has been added to the EIS (Appendix M) which describes a recent Workshop on Studies on Assessment of Potential Risks Associated with Recombinant DNA Experimentation, held at Falmouth, Massachusetts, on June 20-21, 1977.

Essentially, E. coli strain K-12 should not be characterized as a pathogen; and a major advantage of using E. coli instead of other bacteria is the immense knowledge of its genetic makeup and biochemical pathways. Because of this knowledge, well-characterized and precisely located mutations can be introduced into the K-12 strain by standard genetic techniques, to create variants that have specific disabilities precluding their survival in nature.

There is no other bacterium whose characterization even remotely approaches that of E. coli K-12. Two principal advantages for its use in recombinant DNA research are as follows:

- Laboratory strains of E. coli have never been observed to cause disease in laboratory workers.

- The K-12 strain does not carry the surface component required for E. coli to establish itself in humans. Experiments have shown that man must ingest at least a million K-12 organisms to obtain even transient multiplication in the intestine (references 30 and 31 of the Guidelines).

There is more information about E. coli than about any other class of microorganism. The modern sanitation practices and sewage treatment systems that have virtually eliminated from developed countries the once-prevalent epidemics of intestinal pathogens, such as typhoid, cholera, and dysentery, would provide the same barrier against widespread dissemination of E. coli-containing recombinants. Further, conversion of a laboratory strain to pathogenicity is likely to require alterations in many genes; the chance that alteration of a single gene might lead to pathogenicity is extremely remote. For example, it is known that two separate plasmid-coded genes can, together, convert certain strains of E. coli into pathogens. However, these same two plasmids, when inserted into E. coli K-12 do not result in pathogenicity: the recipient K-12 is not capable of inducing disease in susceptible animals. Thus, not only pathogenic genes, but also the nature of the cell carrying those genes, is important in the determination of pathogenicity.

Recognizing the ability of certain plasmid vectors to transmit themselves and any foreign DNA to a recipient host cell other than that used in the laboratory, the Guidelines do not permit use of such plasmids--referred to as "conjugative" plasmids. The possibility that nonconjugative plasmids may themselves be transferred under certain conditions is extensively discussed in the Guidelines (Section III-A). The probability of the latter transfers can be quite low; the data for each case are considered in the certification of EK2 and EK3 host-vector systems. The criteria recently adopted by the Recombinant Advisory Committee for the certification of EK2 host-phage and host-plasmid systems are included in Appendix H of the EIS.

D. No Guidelines but NIH Consideration of Each Proposed Project on an Individual Basis Before Funding

There were no specific comments directed exclusively to this Section.

E. General Federal Regulation of All Such Research

A number of commentators addressed concerns about the scope of the NIH Guidelines and the need for regulation of all recombinant DNA research nationally and internationally [11,12,14,17,29]. Two commentators suggested that a national commission be formed to ensure representation beyond the control of the NIH [12,28]. From the time the Draft EIS was filed in the Federal Register of September 9, 1976, there have been Federal activities directly related to these concerns. The Federal Interagency Committee on Recombinant DNA Research was created to address these and other important public policy issues. The pending legislation directly addresses the need for regulating all recombinant DNA activities. This subsection of the EIS has been expanded to cover these and other activities that have taken place over the last year.

Several commentators were concerned about the extent of actual compliance with the NIH Guidelines by NIH grantees and contractors [20,26]. The Guidelines place responsibility on the investigator and the local institution for initial implementation of the safety standards. Review by the NIH study sections, the NIH Office of Recombinant DNA Activities, and the NIH Recombinant Advisory Committee provides further monitoring. At the local level, as noted before, the Guidelines recommend broad representation on biohazards committees. The NIH in its own intramural program has a biohazards committee to review intramural recombinant DNA experiments. This committee now includes a representative from the local county health department. Of course, if legislation is enacted for the regulation of recombinant DNA activities, the NIH and its grantees or contractors, as well as any others conducting this research, will be subject to such regulation.

Several commentators expressed concern about the shipping practices required for recombinant DNA molecules [26,29,32]. There were two primary concerns. One related to the need for a defined approach or program to deal effectively with an accidental spill during transit, so that any threat to the public health could be minimized. The second concern related to the limited scope of existing Federal regulations governing transport of potentially hazardous microoganisms. Existing regulations apply only to etiologic agents listed by the Public Health Service in Section 72.25 of Part 72, Title 42, Code of Federal Regulations.

The Guidelines require that all recombinant DNA materials (i.e., recombinant DNA molecules, and vectors or hosts containing such molecules), regardless of whether or not they are constructed from an etiologic agent listed in paragraph (c) of 42 CFR 72.25, be contained during shipment in packages that meet the requirements described in the cited Federal Regulations. Appropriate packaging methods are described in detail in Appendix D, Section X of the Guidelines.

The packaging methods have demonstrated containment capability. The methods have been extensively evaluated and shown to prevent leakage to the environment under rough-handling test conditions specified in DOT 49 CFR 173.387 (16, 17) and under severe impact tests (e.g., impact on concrete at 90 miles per hour) (17).

The packaging methods described in the Guidelines have been extensively employed for the shipment of highly infectious etiologic agents. There are no known cases of infectious disease having been transmitted to personnel involved in handling packaged material during shipment (8). The required use of these packaging methods should reduce significantly any potential for leakage of recombinant DNA materials during shipment and for subsequent harm to the public health or environment.

It is true that the labelling requirements specified in PHS 42 CFR 72.25 are to be used only for "viable recombinant DNA materials in which any portion of the material is derived from a listed etiologic agent." This limitation is based on an interpretation that PHS 42 CFR 72.25 precludes the use of the label on materials not containing any portion of a listed etiologic agent.

Since the label cannot be uniformly used at this time, a problem in surveillance during shipment is created for some recombinant DNA materials. The primary benefit of the label is that in the event of damage to the package or leakage during shipment, the Center for Disease Control (CDC) is notified immediately. The CDC would then initiate appropriate action to deal with the particular situation. This action may involve simply providing guidance for decontamination, or may involve more elaborate precautionary procedures, depending on the situation. The response mechanism provided by CDC, however, is not now available for some recombinant DNA materials that may be shipped.

Although the possibility for leakage is remote, a uniform program for labelling and notification of CDC in the event of any problem during shipment is important. The Department of Transportation and the Center for Disease Control are considering amending Federal regulations governing transportation of etiologic agents to extend the coverage of those regulations to a number of presently excluded substances, such as recombinant DNA materials. The NIH will encourage these agencies to require labelling of all recombinant DNA materials.

Some commentators have suggested that the Draft EIS was deficient in not considering the alternative of placing all recombinant DNA molecule research under government monopoly [14, 32]. These suggestions are amorphous and lacking in detail. Such a regime would presumably involve a prohibition of any such research except by the Federal agency to which the monopoly is entrusted. It is not clear whether the commentators contemplate that private individuals might perform such research as contractors or grantees of the agency and, if so, whether the research activities could be performed in private facilities. Suggestions of this kind go far beyond the limited scope of the Guidelines to which the Draft EIS is directed and raise fundamental and extremely broad policy issues. In any event, the NIH is not aware of any facts or circumstances at the present time that would warrant consideration of so extreme an alternative. On the other hand, the NIH and the Interagency Committee intend to monitor developments closely, in order that full consideration may be given to all appropriate alternatives in the light of new knowledge that may emerge from scientific research.

It has also been suggested that the EIS should consider the alternative of placing all recombinant DNA molecule research under an international agency [14, 32]. NIH is not aware of any meaningful precedent for such an action. The practical and political difficulties in bringing about such a structure are so great, and its creation necessarily lies so far in the future, that this possibility does not warrant serious consideration as an alternative to the Guidelines.

A number of commentators were also concerned about regulation of this research outside the United States [23, 32]. Over the past several months, the NIH has maintained close liaison with governmental and nongovernmental organizations abroad involved in activities relevant to the conduct and control of recombinant DNA research. The NIH is committed to the promulgation of safety measures for all such research nationally and internationally, and will cooperate fully with all research and regulatory efforts in this regard. Further, the representative on the Interagency Committee from the United States Arms Control Disarmament Agency has reaffirmed that the use of recombinant DNA molecules for biological warfare is prohibited by the Biological Weapons Convention. As noted previously, a statement on this subject by Ambassador Joseph Martin, Jr., has been included in Section IV-C-4 of the EIS.

An ongoing review of international activities by the Interagency Committee on Recombinant DNA Research, reporting to the Secretary of Health, Education, and Welfare, will help to determine what steps may be warranted by the Government to ensure effective coordination for international safety practices and procedures to govern recombinant DNA activities throughout the world.

VII. ENVIRONMENTAL IMPACT OF THE GUIDELINES

This section has been extensively revised in response to many points raised by the commentators and with a view to clarifying and updating the material generally. An effort was made to provide additional information, not only to answer substantive questions but also to provide more background so that readers would be better able to understand this section and to formulate their views.

A. Impact of Issuance of NIH Guidelines

Many commentators challenged the adequacy of the Guidelines to protect the public and the environment from alleged hazards of recombinant DNA research. This section of the EIS now states explicitly that a totally risk-free situation was not the aim of the Guidelines. The following subsection addresses this issue.

1. Impact on the Safety of Laboratory Personnel and on the Spread of Possibly Hazardous Agents by Infected Laboratory Personnel

One commentator questioned whether the containment requirements of the Guidelines were adequate and based on firm knowledge of the risks and well-established principles of classification and containment [12]. Several recommended that documentation be provided to support the assertion that "potentially harmful effects from research with high-risk recombinant DNA molecules should be extremely unlikely, given strict adherence to the NIH Guidelines" [3, 12, 17, 20, 26]. One commentator also questioned the advisability of (a) permitting P3-level research to be conducted in class III biological safety cabinets in facilities where directional air flow and exhaust air capabilities were not present and (b) not requiring secondary decontamination of waste water for P1, P2, and P3 levels of physical containment [20]. Other commentators felt that the requirements for P2, P3, and P4 facilities should be made more stringent [12, 38]. In view of these kinds of questions, the text of this subsection of the EIS has been radically revised to document the adequacy of the containment requirements and to show the bases on which judgments in this regard have been made. For example, the discussion of past successes in conducting biohazards programs has been much expanded, in response to one commentator who explicitly criticized the Draft EIS for its lack of such documentation [20].

The physical containment safeguards required by the NIH Guidelines are based on the accumulated knowledge and experience in the area of biological safety that has been acquired over the last three decades. The EIS draws extensively on the experience at Fort Detrick in the containment of serious human pathogens.

An examination of the experience with laboratory-acquired infections at Fort Detrick provides a basis for demonstrating the value of biological safety cabinets. The frequency of infections for four periods of operation are summarized in a text table of this EIS. The periods characterize four levels of physical containment that are similar to levels P1, P2, P3, and P4 with respect to required use of biological safety cabinets. That is, in the period 1943-45 all research was conducted on the open bench (9). Class I and class III biological safety cabinets were introduced at Fort Detrick after 1950 and were assigned on a risk-priority basis to programs where considerable aerosol exposures were likely to occur. After 1960 class I and class III biological safety cabinets were generally available for most research programs. The example period 1960-69 provides the experience for the only program at Fort Detrick that was conducted in accordance with the requirements for P4-level physical containment (10). It is this 10-year experience, where only one infection occurred, on which our confidence for the capability of P4 physical containment is based.

An absolute correlation between the reduction in rate of laboratory-acquired infections and the use of biological safety cabinets is admittedly difficult to establish. During the 1960-69 research at Fort Detrick, a variety of etiologic agents were employed and the use of biological safety cabinets was dependent on their availability and assignment. Also, the the introduction of effective vaccines for anthrax in 1954, tularemia in 1959, and Venezuelan encephalitis in 1962 may have influenced this experience [2]. (It should be noted that the detection of an increase in antibody titre, regardless of immunization status, was recorded as a laboratory infection.) Nevertheless, the performance of biological safety cabinets in containing test aerosols attests to their importance in reducing potential inhalation exposures.

It is recognized that human error and accidents contribute to laboratory-acquired infections [3,12,17]. The Fort Detrick experience demonstrates that even the best possible safety equipment cannot prevent self-infection through human error. The one infection that occurred during the period 1960-69 in Building 1412B was caused by self-inoculation with a contaminated needle through the attached glove of a class III cabinet. Pike (11) has shown that most accidents that have been correlated with laboratory-acquired infections involve (a) the use of needle and syringe, (b) overt contact with spills, (c) injury from contaminated broken glassware, (d) improper use of pipettes, and (e) bites and scratches from infected animals. Occurrence of these accidents, however, can be significantly reduced by diligent adherence to the NIH Guidelines.

The text includes further description and evaluation of class III cabinets.

The NIH Guidelines require that waste water from a P4 facility be collected and sterilized before being discharged to the sanitary sewer. This is a secondary precaution to prevent the release of a contaminant to the environment in the event of an accidental escape of microorganisms from the primary barrier system (i.e., class III biological safety cabinet). For experiments of less hazard-potential (i.e., P1, P2, and P3), the NIH Guidelines require decontamination of all contaminated liquid and solid materials before disposal. Secondary sterilization, however, is not required for these materials. In the review by Wedum (10), no evidence was found that this additional precaution is needed.

2. Impact on the Environmental Spread of Possibly Hazardous Agents

There is no supporting epidemiological experience which suggests that the research conducted at Fort Detrick was hazardous to the public. Although there was a total of 423 laboratory-acquired infections over the 25 years of Fort Detrick's operations, during which a variety of primary containment conditions were employed (P1 to P4), no member of the general public was ever infected as a result of research at Fort Detrick (12).

Experience with highly infectious microorganisms does not indicate a need to provide more stringent facility safeguards for the P3 and P4 levels than those required by the NIH Guidelines. Wedum (10) stated: "As far as biohazard outside the building is concerned, most secondary barriers are more for reasons of public relations than for anything else, except for pilot plants or other large-volume production, experimental aerosols, use of tick or insect vectors, and agents capable of spread to animal or plant food supply. This view assumes that known infectious liquids, solids, animals, and animal wastes are decontaminated before disposal, as has long been standard practice in all microbiological laboratories."

3. Cost Impact

Several commentators also requested specific cost estimates for preparing facilities to accommodate recombinant DNA research [13, 20]. The text has been expanded to provide such information.

4. Secondary Impacts

No comments were addressed to this Section.

B. Impact of Experiments Conducted Under the Guidelines

1. Possible Undesirable Impacts

It is apparent in reading several of the comments on the Draft EIS that the description of a theoretical approach to evaluating the probability of occurrence of a hazard from a given recombinant DNA experiment was confusing. That Section has been rewritten in the interest of clarity. As pointed out, it is not necessary to know the probabilities of all the individual events leading to a hazardous situation in order to obtain a useful estimate of the probability of its occurrence.

Commentators noted the reference in the Draft EIS to NIH research designed to improve our ability to determine the risk probabilities of recombinant DNA work [20, 30]. The revised EIS identifies this research as that to be undertaken at the Frederick Cancer Research Center. In addition, NIH has initiated a contract-supported program aimed at significantly reducing presumed biohazards of recombinant DNA research through the development of safer hosts and vectors. The revised EIS also contains a new appendix, H, on the procedure and rationale for certification of EK2 host-vector systems.

Two commentators faulted the Draft EIS for not covering long-range evolutionary consequences in this section [2, 30]. Totally new material has been added to address the issue.

In addition, quotations were introduced from recent public articles representing scientists' views on the issue of potential hazard.

2. Beneficial Impacts of Recombinant DNA Research

This section of the EIS has been updated.

CONCLUSION

Many of the issues raised by the commentators on the Draft Environmental Impact Statement were previously considered in the development of the NIH Guidelines for Research Involving Recombinant DNA Molecules. In drafting an impact statement, however, the NIH has given further consideration to these issues and has provided an opportunity for the public to comment from an environmental perspective. This perspective has been extremely helpful in NIH's review of policies and procedures on recombinant DNA research.

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